TABLE C-1 Continued

Reference	Demographics	Group	Questions
Marriott, 2007	April–August 2005 (n=16,146) Response rate overall: 51.8%	Active duty military (worldwide) All four branches and pay grades Army (n=3,636) Navy (n=4,626) Marine (n=3,356) Air Force (n=4,627)	DoD Survey of Health Related Behaviors Among Military Personnel plus added questions on diet, DS, CAM

Findings: Usage	Other Findings	Authors' Conclusions and Study Limitations
Supplement use (last 12 months): Any: 60.3% Multivitamin: 45% Individual vitamin/mineral: 26.9% Antioxidants: 20.8% Bodybuilding: 20.5% Herbal: 11.7% Weight loss: 18% Joint health: 8.5% Performance: 8.4% Other types: 9.1% **Frequency of use:** Once a month: 8.3% Once a week: 7.6% Every other day: 7.7% Once a day: 30.7% 2 times or more a day: 12.8% **Reporting use to military personnel:** To doctor: 36.6% To nurse/physician assistant: 21.7% Indicated on health record: 12.8% Women: 62.5% Men: 45.2% Reporting increases with age **Reporting to doctor by military branch:** Air Force: 49.5% Navy: 32.6% Army: 31.5% Marine: 25.3%	**Reasons for use:** Supplement diet Improve health Improve mental health Improve cognitive function Improve physical performance Increase muscle mass Lose weight Specific health problems **Information sources:** Personal contacts: 47.9% Print media: 29.1% Friends/family: 24.8% Multimedia nonprint: 23% Health professionals: 17.3% Sales store associates: 5.7%	**Conclusions:** • Nutrient supplements provided through military health care • Diet: <10% eat three or more servings of fruit and vegetables per day • Women in the Navy highest reported fruit (10.7%) and vegetable (12.9%) of three or more servings per day • Military dining services may represent an opportunity for increased communication about healthy diet and dietary supplement use Study also includes information on: Bodybuilding supplements, performance-enhancing supplements, and weight-loss supplements by pay grade, gender, age, education, BMI, activity, smoking, drinking, and supplement use overall by gender, military branch Author suggestions: analysis of specific information on exact supplements used with estimates of nutrient intake from food and supplements

continued

TABLE C-1 Continued

Reference	Demographics	Group	Questions
Thomasos, 2007	2006 (spring/summer) (n=10,985)	Air Force enlisted and officers	Adapted from the U.S. Army Center for Health Promotion and Preventive Medicine Dietary Supplement Questionnaire "Have you ever used any type of dietary supplement?"

NOTE: BMI=body mass index; CAM=complementary and alternative medicine; DS=dietary supplement; PA=physical activity.

	Findings: Usage	Other Findings	Authors' Conclusions and Study Limitations
	Supplement use: Currently using: 38% Past use only: 31% Never used: 31% **Most common supplements used** (5 or more times/wk): Multivitamin Multimineral Creatine Glutamine Vitamin C Caffeine Calcium Protein powders Fish oils Hydroxycut Chondroitin NO_2	**Primary reasons for use** (from most to least commonly reported): Promote health, weight loss, strength, lean muscle mass, stamina, fatigue reduction, cognition/alertness, memory, other **Most common supplement used:** (1) Senior enlisted: for weight loss (2) Junior enlisted/officers: for strength and lean body mass gains **Sources of information** (most to least commonly reported): Magazines, friends/family, books/journals, internet sites, health care providers, tv/radio/newspaper, store salesperson **Supplement purchase:** Commissary/PX: >40% Other on-base stores: ~25% Off-base stores: ~24% Internet/mail order: <10% **Adverse effects** (most to least commonly reported): Palpitations, anxiety, dehydration, nausea/vomiting, chest pain, dizziness/confusion, abdominal pain, tremors, diarrhea, muscle pain, numbness/tingling, breathing problems, heart attack, blood in urine, heat injury, visual disturbances, fainting	**Conclusions:** • Most U.S. Air Force members using DS • >60% DS bought on base • Most spend between \$11–\$50/mo • Most members do not report having adverse effects on this survey • Adverse effects are *not* usually reported to health care providers • Education on DS is needed **Additional information:** • Supplements were categorized into "like types" • Study also provides information on supplement use by: Occupation Grade (O1–O6 and E1–E9)

TABLE C-2 Surveys on Use of Dietary Supplements by Military Personnel

Reference	Demographics	Group	Questions	Findings: Usage
Arsenault and Kennedy, 1999	n=2,215 males, Average age: 25 (18–47 y) Response rate: 99%	Men entering U.S. Army Special Forces and Ranger training schools	Use of vitamins, minerals, pro-performance or other supplements	**Supplement use:** Past and/or present: 85% **Current use:** Any kind: 64% Ergogenics: 29% Multivitamin: 37% Vitamin C: 20% Creatine: 18% Ginseng: 9% **Daily use:** Any kind: 35% Ergogenics: 14%
Bovill et al., 2000[a]	n=152 male Average age: 31 y Response rate not known	U.S. Army Special Forces	Surveys asked about nutrition, supplement use, demographics, and health habits	**Supplement use:** 86% occasionally **Frequency:** Sports drinks: 66% Sports bars: 43% Multivitamins: 42% Vitamin C: 22% Protein powder: 22%

Findings: Motivation	Other Findings	Authors Conclusions and Study Limitations
Use for: General health, performance enhancement, interest in preventing infectious diseases, physical performance, and wound healing	• Use is higher than in general population of young men (39% use them occasionally) • Supplement use associated with higher scores for Army physical fitness tests, daily exercise, weight lifting, and nonsmokers • Not associated with age, ethnicity, BMI, chewing tobacco, or alcohol use	**Conclusions:** • Military population is at risk for potential adverse effects of inadequate use of DS, including abrupt cessation when deployed in operations or entering training • Presence of DS on military bases and discontent with food might result in higher use of DS **Recommendations:** • Further studies of benefits and risks are necessary • Routine one-size-fits-all advice should not be given • OK to treat deficiencies but questions about performance effects • Military health care professionals should be well informed about risks and benefits
General health: 63% Performance: 20%	• Supplement use associated with frequency of strength training • Not associated with age or habitual exercise • Information on nutrition from: Magazines/newspapers/books: 76% Friends: 54% Radio/TV: 34% Physicians/nurses: 33% Internet: 30%	

continued

TABLE C-2 Continued

Reference	Demographics	Group	Questions	Findings: Usage
Bovill et al., 2003	n=157 male (119 Special Forces, 38 non-Special Forces) Response rate: 89%	U.S. Army Special Forces and support soldiers (non-Special Forces)		**Supplement use:** Current: 87% **Specific use:** Similar to above (Bovill et al., 2000)

Findings: Motivation	Other Findings	Authors Conclusions and Study Limitations
A majority (64%) of soldiers incorrectly believed that protein is used for energy for short-term athletic events, 58% believed that vitamins provide energy	• Supplements used by more Special Forces (90%) than non-Special Forces (76%) • Supplement use associated with more frequent exercise and greater nutrition knowledge • Not associated with age, weight, ethnicity • Information on nutrition similar to above (Bovill et al., 2000)	**Conclusions:** Possible trend in increased use of supplements in military

continued

TABLE C-2 Continued

Reference	Demographics	Group	Questions	Findings: Usage
Brasfield, 2004	n=874 750 men 124 women Average age: 24.9 y (17–49 y) Response rate: 64%	Enlisted U.S. Army, active duty Representative sample of soldiers	Use of supplements and motivation	**Supplement use:** 60.9% **Frequency:** Multivitamin: 56% Vitamin C: 28% Creatine: 23% Ephedra: 21% Ginseng: 21% Calcium: 20% Vitamin E: 15% Vitamin A: 13% Iron: 13% Garlic: 12% **DS users consumed:** Three or more DS: 53% Two DS: 22% One DS: 25%
Deuster et al., 2003	n=38 Average age: 25 y (18–40 y) Response rate: 100%	U.S. Army Rangers	Nutrient, alcohol, DS intake, and physical activity level	**Daily supplement use:** 81.5% **Most common:** CHO/electrolyte fluids Protein powder: 24% Creatine: 13% Ephedrine: 13% Also reported use of ginseng, glutamine, vitamins/minerals

Findings: Motivation	Other Findings	Authors Conclusions and Study Limitations
General health, performance enhancement, prevent illness	**Source of information** (in order of frequency): Other (friends, family, etc.) Magazines Store salesperson Internet Doctors Books TV More females than males consumed a DS; however, more men consumed creatine, ginseng, and garlic No association with aerobic exercise frequency **Adverse events:** 18% Palpitations: 46% Dizziness/confusion: 30% Tremors: 26% Abdominal pain: 24% Numbness/tingling extremities: 16% Loss of consciousness: 4%	**Conclusions:** High number of adverse effects that might be attributed to DS use **Recommendations:** • Use of some DS should be discontinued prior to undergoing surgery • Health care screening • Routine assessment • Education • Further surveys recommended **Limitations:** • Self-reporting might result in misunderstanding and misreporting • Generalizations to general population are not possible • Categorization of DS Stores on military bases specifically to sell DS, in 2004, 92 stores worldwide on military installations
		Conclusions: Use of supplements is not necessary based on the dietary intakes of protein by the Rangers

continued

TABLE C-2 Continued

Reference	Demographics	Group	Questions	Findings: Usage
Johnson et al., In press	n=294 Average age: 23 y (survey was conducted in 1999) Response rate: 40%	U.S. Army Rangers	Use of supplements; potential factors associated: age, participation in competitive or recreational athletics, weight training; sources of nutritional information	**Supplement use:** 56% **Frequency:** Whole protein: 62% Creatine: 46% Thermogenics: 44% Anabolic steroids: <2%
McGraw et al., 2000	n=367 Average age: 22 y Response rate not known	U.S. Army Rangers	Use of supplements and associated factors	**Supplement use:** 36% **Frequency:** Creatine: 19% Multivitamin/ multimineral: 16% Protein/Amino acids: 14% Vitamin C: 7% Sport bars: 6%
McPherson and Schwenka, 2004	n=291 Average age: 39 y (18–83 y) Response rate: 73%	U.S. soldiers, retirees, spouses in military hospital	Use of complementary and alternative medicine (CAM)	Massage and supplements were most commonly used **CAM use:** Active duty: 72% Retiree: 85% Family: 89% **Supplement use:** Herbal supplements: 36% Nutritional food supplements: 36%

Findings: Motivation	Other Findings	Authors Conclusions and Study Limitations
	• No difference in age of user compared to nonuser • Supplement use associated with recreational activities, weight training • Source of information: Other soldiers: 59% Fitness magazines: 46% Internet: 18% Nutritionist: 8% Unit surgeon: 6%	**Conclusions:** • Soldiers consume ergogenics at the same rate as other athletic populations • Supplement use is inversely correlated to nutritional knowledge **Recommendation:** Further education of unit surgeons **Limitation:** Self-exclusion of soldiers with higher usage might result in low response and apparently lower supplement use level
	• Supplement use lower than general male population (42%), elite athletes (59%), or U.S. Army Special Operations candidates (64%) • Supplement use associated with frequency of strength training • Not associated with smoking or aerobic training.	
Pain, stress, anxiety, depression, weight loss	81–98% thought the treatment was effective	**Conclusions:** • Health providers need to become educated in CAM therapies • Further studies needed **Limitation:** Geographical area might have biased the results, Western region (increased CAM, more providers)

continued

TABLE C-2 Continued

Reference	Demographics	Group	Questions	Findings: Usage
Schneider et al., 1998	n=91 Response rate not known	Naval Sea, Air, Land (SEAL) personnel		**Supplement use:** 78% Using more than one supplement concurrently: 4–9 DS: 32% 3 DS: 34% 2 DS: 18% 1 DS: 16%
Sheppard et al., 2000	n=229 (133 military) Response rate: 40%	U.S. civilian and military health clubs	Use of creatine and other supplements	**Creatine use:** 12.2 g/d for 40 wk **Military supplement use:** Vitamin: 65% Mineral: 47% Protein: 45% Creatine: 29% Herbal: 21% Androstenedione: 13% Hydroxy-β-methyl-butyrate (HMB): 10% Anabolic/androgenic steroids: 3%

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[a] Some of the findings from Bovill et al. (2000) and Bovill et al. (2003) are virtually the same and might come from the same study.

Findings: Motivation	Other Findings	Authors Conclusions and Study Limitations
Increase muscle mass, strength, and power; provide energy; improve general health		Notes: Navy, concurrent supplements
	• Supplement use associated with resistance training goal of strength • Creatine use associated with male gender, goal of strength training, lower frequency and duration of aerobic training, use of protein, andro/DHEA, and HMB • Information on creatine from: Popular media: 69% Physicians: 14% Dieticians: 10%	Conclusions: • Concerns: creatine use with other anabolic supplements • Popular magazines as main source of information • Education and access to information of users is critical • 45% of current creatine users reported adverse effects: gastrointestinal, muscle cramping/spasms, dehydration

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Appendix D

Case Studies

THE CASE OF DEHYDROEPIANDROSTERONE: DECISIONS FOR ACTION

Dehydroepiandrosterone (DHEA) was selected for a review of safety and efficacy because, as the committee compiled data about the use of dietary supplements by military personnel, anabolic supplements or bodybuilding supplements were highlighted as one of the categories of dietary supplements that were most popular. DHEA is a steroid compound that is also popular in the civilian population because of its alleged effect in increasing muscle mass and enhancing physical performance. It is no surprise then that, performance enhancement being one of the main reasons military personnel cite for taking dietary supplements, DHEA has become popular among military members. DHEA was among the top 10 dietary supplements used at least once a week by Rangers (7 percent) and Special Forces (6 percent) in surveys conducted in 1999 and 2000, respectively (Lieberman et al., 2007). In another survey comparing civilian and military use of dietary supplements among members of health clubs, as many as 13 percent of military personnel were using DHEA (Sheppard et al., 2000). When asked by health care providers about "bodybuilder supplement use," 6.6 percent of military members reported using them (Jaghab, 2007); DHEA may have been among them. In addition, the most recent U.S. Department of Defense (DoD) Survey of Health Related Behaviors Among Military Personnel (Marriott, 2007) found that as many as 20.5 percent of military personnel used bodybuilder supplements within the last 12 months. As a variety of sources suggested a high level of use, the committee initiated a review of DHEA safety and efficacy. The committee searched for literature reviews (Figures 4-1 and 4-2) conducted over the previous 10 years as well as more recent original studies not included in reviews. In addition, a search was conducted for articles specifically designed to signal safety or performance effects of critical importance to the military.

Surprisingly, the popular view that DHEA increases muscle mass and therefore might improve performance appears to be based largely on the findings of a 1998 paper that had significant methodological limitations (Morales et al., 1998). The various reviews of the literature addressing efficacy indicate that there is little substantiation for such a performance claim. There is a gender-specific effect on blood testosterone that perhaps merits further research to determine effects of DHEA on lean tissue and bone density gain in women during resistance training. Some reviews found that the use of DHEA by women increases testosterone concentration. Other reviews evaluated suspected benefits on cognition, mood, and bone strength from consuming DHEA. A search for original articles on studies that include situations or conditions of particular relevance for the military yielded no result. Based on Table 4-2 and the findings from literature reviews, the committee agrees that there is a low level of benefit to be gained by military personnel from using DHEA. Reviews highlighted adverse androgenizing effects experienced by women, and other minor effects such as facial acne or increased sebum production; there was no adverse effect identified that would decrease the readiness of military personnel. There was some theoretical increased risk of cardiovascular disease in women due to the reduction in high-density lipoprotein noted in some studies.

Although some drugs are known to either increase or decrease blood DHEA, there were no reports that supplementary DHEA affected the action of most drugs. Other prescription steroid hormones (e.g., testosterone analogs, estrogen) may be exceptions; it is possible that DHEA consumption could affect the metabolism of those drugs.

One long-term theoretical but critical adverse effect uncovered during the safety reviews is the potential association of DHEA levels in blood with a higher risk of breast cancer seen in various epidemiological studies. The potential for hepatic neoplasia was also suggested by results from a review of animal studies. This potential adverse effect is serious enough that, although a cause and effect could not be established from those studies, a high level of concern was determined for DHEA (Box 4-1).

The military should decide on the course of action based on the high level of concern and low benefit derived from its use. A course of action might be to do the following:

- Follow up with the research community to determine whether the equivocal animal data related to neoplasia are translated to humans and to monitor future research on either safety or benefits to determine if it needs to be reclassified in the future, which would likely lead to different management actions. Research on DHEA should be monitored to determine if it should be reclassified as future research unfolds.

- Develop an outreach strategy to educate military members about the high risks and low benefits of using DHEA by
  - including DHEA in a list of dietary supplements to avoid. Recommend the use of alternative products (e.g., creatine, beta-hydroxy-beta-methylbutyrate [HMB]) or strategies (modification of resistance training regime, increase of energy intake) that might provide similar desired effects;
  - informing military health care providers, fitness trainers and therapists, registered dietitians, nutritionists, commanders, and other educators about the risks and benefits of using DHEA and recommend alternative products or foods; and
  - monitoring use and potential adverse effects among military personnel.

THE CASE OF EPHEDRA: DECISIONS FOR ACTION

Ephedra (*Ephedra sinica* Stapf and other ephedrine-containing *Ephedra* species) was selected for a review of safety by the committee for various reasons. First, due to the severe adverse effects reported, the sale of ephedra in dietary supplements has been banned in the United States since 2004 (Rados, 2004); it is therefore the first and only dietary supplement that has been banned since the Dietary Supplement Health and Education Act was implemented (the ephedra alkaloids ephedrine and pseudoephedrine, however, are allowed to be sold as over-the-counter medications in the United States). A study by Deuster et al. (2003) reported that 13 percent of the U.S. Army Rangers surveyed used ephedrine. Similarly, a high percentage (21 percent) of ephedra users were calculated from a self-reporting questionnaire distributed among U.S. Army active duty personnel (Brasfield, 2004). These surveys, however, were conducted prior to the ephedra ban in the United States; the impact of the ban on the use of ephedra and its alkaloids is not known. A survey focusing on supplement use that was distributed among Army health care providers revealed that 5.2 and 7.7 percent of soldiers reported use of ephedra to their physicians or other health care personnel, respectively (Jaghab, 2007). Although these numbers might not be representative of the military population, they do raise safety concerns about the use of ephedra. The odds of adverse events from misuse of over-the-counter medications containing ephedra alkaloids might be small but continue to be of concern. Also, botanicals that are chemically similar to ephedra and might mimic its effects are still available in the market.

Although initially the military had considered ephedra among the dietary supplements likely to be efficacious and of interest, defense applications for use of ephedra were never developed by The Technical Cooperation Program (TTCP) panel[1] because of safety concerns (Lieberman et al., 2007). These safety concerns and its use among military personnel prompted a safety evaluation of ephedra.

The committee initiated a safety review by applying Figures 5-1 and 5-2 (see Chapter 5). An initial search for reviews of ephedra was carried out in appropriate databases such as PubMed, Napralert, Toxline, SciFinder, UIC, and Company Digital Libraries. Among the terms used in the search were *ephedra* and Latin binomials, *healthy*, *performance*, *ergogenic*, *memory*, *interactions*, *adverse*, *toxicity*, and *infection*. The review only focused on perceived benefits such as increased weight loss and performance enhancement as relevant benefits for military personnel. A few studies demonstrate a statistically significant weight loss using ephedra versus placebo. Most studies, however, showed a weight loss of only 0.6–0.8 kg per month using ephedra, or 1.0 kg with ephedra-caffeine combinations. The committee concluded that these effects are not clinically relevant. Moreover, there are no clinical studies with long-term data. Likewise, clinical studies with ephedra alkaloids have not been shown to result in significant improvements in performance for the specific modalities tested. However, combinations of ephedrine HCl (synthetic ephedrine) and caffeine seem to enhance various measurements of performance.

During clinical trials it was noted that the risk of adverse events increased two- to fourfold and that the adverse effect profile of ephedra was primarily related to serious cardiovascular effects, from palpitations to tachycardias and strokes. Although most adverse effects are relevant to the general population, some of them, such as psychosis, vision impairment, dehydration, or muscle failure, would specifically present heightened risks for military personnel. Interaction with sympathomimetic drugs as well as the occurrence of palpitations should be of concern. Some of the adverse events (e.g., psychosis, increased heart rate and blood pressure, myocardial infarction, arrhythmias) were also seen in studies when ephedra and caffeine were provided in combination.

The committee concluded that the use of ephedra (and related alkaloids) presents a high level of concern. With only moderate potential for benefits and the high level of concern, this committee supports the current ban on ephedra use. Military leadership might decide to take the following actions on ephedra and its alkaloids, particularly directed toward populations that might use performance enhancers, such as Rangers or Special Operations forces:

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[1] The TTCP panel is an international panel of military scientists whose mission is to conduct research, share information, and write papers on performance-enhancing treatments for potential operational use.

- Develop an outreach strategy to educate soldiers about the high risks and low benefits of using ephedra and its alkaloids by
  - including ephedra and its alkaloids in outreach materials listing dietary supplements to avoid. Recommend the use of alternative products (e.g., creatine, HMB) or strategies (modification of resistance training regime, increase of energy intake) that might provide similar desired effects;
  - informing military health care providers, fitness trainers and therapists, registered dietitians, nutritionists, commanders, and other educators about the risks and benefits of using ephedra and its alkaloids and recommend alternative products or foods; and
  - monitoring use and potential associated adverse effects among military personnel.

THE CASE OF MELATONIN: DECISIONS FOR ACTION

The committee's interest in melatonin originated from its potential value for use by military personnel as a sleep enhancer and for reentrainment following rapid deployment across time zones (Lieberman et al., 2007). Although melatonin was not reported as being used in any of the military surveys reviewed, melatonin is being used at a high rate as a dietary supplement in the general population. This committee anticipates that in the future, military personnel might be taking melatonin to achieve circadian reentrainment or to improve sleep; therefore, the committee selected melatonin as being of interest to the military and supports a review of safety and efficacy before decisions about its value for military personnel are made.

Melatonin is a hormone secreted in the brain by the pineal gland and also reportedly found in a number of plants. It has widespread effects in the body, many of them poorly understood. Endogenous secretion of melatonin is believed to help maintain internal circadian synchrony among organ systems throughout the body. Exogenous melatonin is available over the counter in the United States. Literature searches conducted by the committee focused on ingestion of melatonin for inducing diurnal sleep in healthy adults, for improving nocturnal sleep in persons with insomnia, and for circadian reentrainment (e.g., for jet lag or night-shift work). The searches were conducted in Thomson ISI and PubMed. There are numerous published clinical studies and experiments. This committee reviewed the findings from three recent reviews (Arendt and Skene, 2005; Morin et al., 2007; Wagner et al., 1998) and three meta-analyses (Brzezinski et al., 2004; Buscemi et al., 2005, 2006). The committee concluded that there is moderate potential for benefits (very modest evidence of improvement of sleep, but moderate to good evidence of circadian reentrainment under controlled conditions).

Mild adverse effects that might affect military performance have been identified, such as drowsiness, core body heat loss, and gastrointestinal distress (e.g., nausea); serious adverse effects were not found. Putative synergistic effects of exogenous melatonin with sedative hypnotics were not found. Given the moderate concern and moderate potential for benefits of exogenous melatonin, the military leadership could initiate the following activities:

- Follow up with the scientific community conducting research on the effects of melatonin for sleep and circadian reentrainment during operations in environments inconducive to sleep, to determine if melatonin has advantages over sedative-hypnotics that have carryover effects on performance.
- Develop an outreach strategy to educate military members, military health care providers, fitness trainers and therapists, registered dietitians, nutritionists, and commanders about the potential interaction of melatonin with sedative-hypnotic medications and the potential for increased heat loss.

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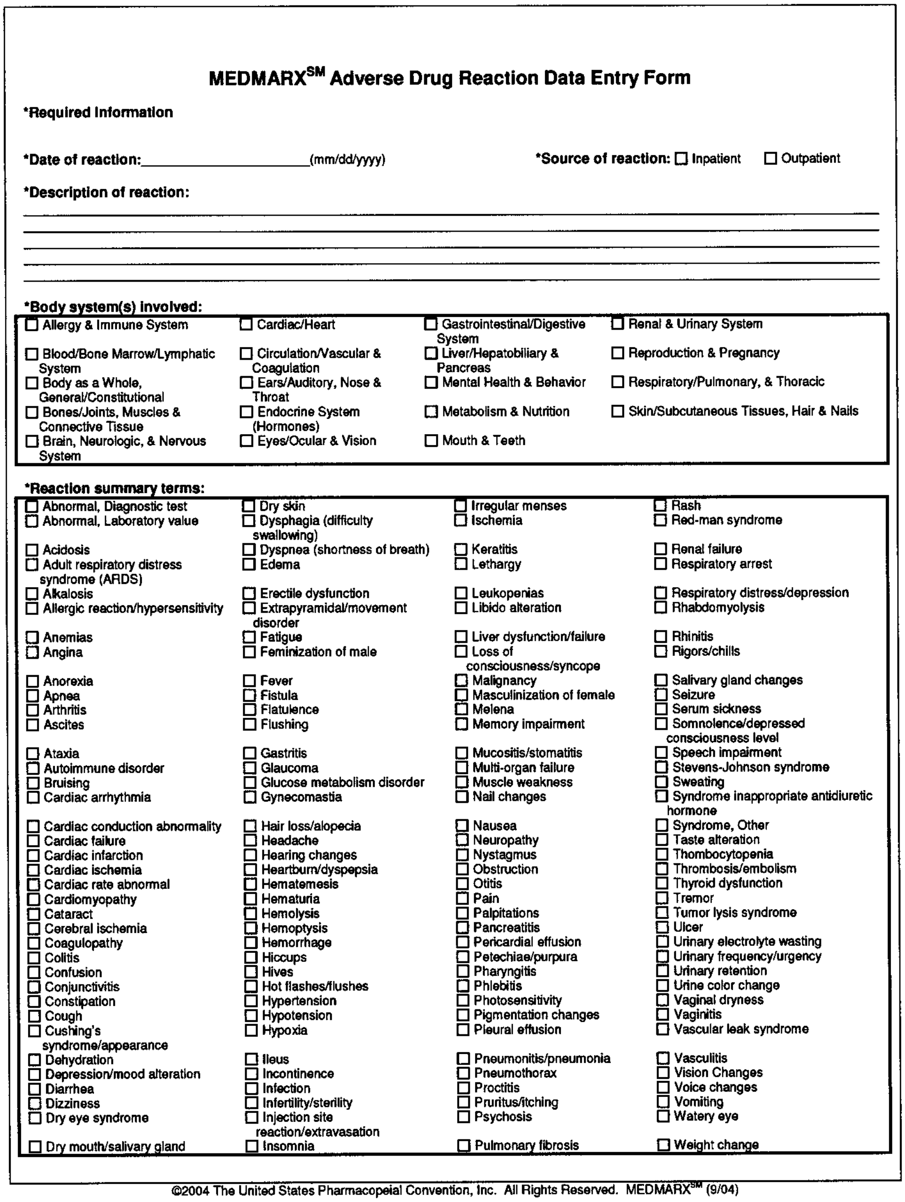
Appendix E

Adverse Event Reporting Forms



MEDMARX^{SM} Adverse Drug Reaction Data Entry Form

*Required Information

*Date of reaction:____________________(mm/dd/yyyy)

*Source of reaction: ☐ Inpatient ☐ Outpatient

*Description of reaction:

____

____

____

____

____

*Body system(s) involved:

- ☐ Allergy & Immune System
- ☐ Blood/Bone Marrow/Lymphatic System
- ☐ Body as a Whole, General/Constitutional
- ☐ Bones/Joints, Muscles & Connective Tissue
- ☐ Brain, Neurologic, & Nervous System
- ☐ Cardiac/Heart
- ☐ Circulation/Vascular & Coagulation
- ☐ Ears/Auditory, Nose & Throat
- ☐ Endocrine System (Hormones)
- ☐ Eyes/Ocular & Vision
- ☐ Gastrointestinal/Digestive System
- ☐ Liver/Hepatobiliary & Pancreas
- ☐ Mental Health & Behavior
- ☐ Metabolism & Nutrition
- ☐ Mouth & Teeth
- ☐ Renal & Urinary System
- ☐ Reproduction & Pregnancy
- ☐ Respiratory/Pulmonary, & Thoracic
- ☐ Skin/Subcutaneous Tissues, Hair & Nails

*Reaction summary terms:

- ☐ Abnormal, Diagnostic test
- ☐ Abnormal, Laboratory value
- ☐ Acidosis
- ☐ Adult respiratory distress syndrome (ARDS)
- ☐ Alkalosis
- ☐ Allergic reaction/hypersensitivity
- ☐ Anemias
- ☐ Angina
- ☐ Anorexia
- ☐ Apnea
- ☐ Arthritis
- ☐ Ascites
- ☐ Ataxia
- ☐ Autoimmune disorder
- ☐ Bruising
- ☐ Cardiac arrhythmia
- ☐ Cardiac conduction abnormality
- ☐ Cardiac failure
- ☐ Cardiac infarction
- ☐ Cardiac ischemia
- ☐ Cardiac rate abnormal
- ☐ Cardiomyopathy
- ☐ Cataract
- ☐ Cerebral ischemia
- ☐ Coagulopathy
- ☐ Colitis
- ☐ Confusion
- ☐ Conjunctivitis
- ☐ Constipation
- ☐ Cough
- ☐ Cushing's syndrome/appearance
- ☐ Dehydration
- ☐ Depression/mood alteration
- ☐ Diarrhea
- ☐ Dizziness
- ☐ Dry eye syndrome
- ☐ Dry mouth/salivary gland
- ☐ Dry skin
- ☐ Dysphagia (difficulty swallowing)
- ☐ Dyspnea (shortness of breath)
- ☐ Edema
- ☐ Erectile dysfunction
- ☐ Extrapyramidal/movement disorder
- ☐ Fatigue
- ☐ Feminization of male
- ☐ Fever
- ☐ Fistula
- ☐ Flatulence
- ☐ Flushing
- ☐ Gastritis
- ☐ Glaucoma
- ☐ Glucose metabolism disorder
- ☐ Gynecomastia
- ☐ Hair loss/alopecia
- ☐ Headache
- ☐ Hearing changes
- ☐ Heartburn/dyspepsia
- ☐ Hematemesis
- ☐ Hematuria
- ☐ Hemolysis
- ☐ Hemoptysis
- ☐ Hemorrhage
- ☐ Hiccups
- ☐ Hives
- ☐ Hot flashes/flushes
- ☐ Hypertension
- ☐ Hypotension
- ☐ Hypoxia
- ☐ Ileus
- ☐ Incontinence
- ☐ Infection
- ☐ Infertility/sterility
- ☐ Injection site reaction/extravasation
- ☐ Insomnia
- ☐ Irregular menses
- ☐ Ischemia
- ☐ Keratitis
- ☐ Lethargy
- ☐ Leukopenias
- ☐ Libido alteration
- ☐ Liver dysfunction/failure
- ☐ Loss of consciousness/syncope
- ☐ Malignancy
- ☐ Masculinization of female
- ☐ Melena
- ☐ Memory impairment
- ☐ Mucositis/stomatitis
- ☐ Multi-organ failure
- ☐ Muscle weakness
- ☐ Nail changes
- ☐ Nausea
- ☐ Neuropathy
- ☐ Nystagmus
- ☐ Obstruction
- ☐ Otitis
- ☐ Pain
- ☐ Palpitations
- ☐ Pancreatitis
- ☐ Pericardial effusion
- ☐ Petechiae/purpura
- ☐ Pharyngitis
- ☐ Phlebitis
- ☐ Photosensitivity
- ☐ Pigmentation changes
- ☐ Pleural effusion
- ☐ Pneumonitis/pneumonia
- ☐ Pneumothorax
- ☐ Proctitis
- ☐ Pruritus/itching
- ☐ Psychosis
- ☐ Pulmonary fibrosis
- ☐ Rash
- ☐ Red-man syndrome
- ☐ Renal failure
- ☐ Respiratory arrest
- ☐ Respiratory distress/depression
- ☐ Rhabdomyolysis
- ☐ Rhinitis
- ☐ Rigors/chills
- ☐ Salivary gland changes
- ☐ Seizure
- ☐ Serum sickness
- ☐ Somnolence/depressed consciousness level
- ☐ Speech impairment
- ☐ Stevens-Johnson syndrome
- ☐ Sweating
- ☐ Syndrome inappropriate antidiuretic hormone
- ☐ Syndrome, Other
- ☐ Taste alteration
- ☐ Thombocytopenia
- ☐ Thrombosis/embolism
- ☐ Thyroid dysfunction
- ☐ Tremor
- ☐ Tumor lysis syndrome
- ☐ Ulcer
- ☐ Urinary electrolyte wasting
- ☐ Urinary frequency/urgency
- ☐ Urinary retention
- ☐ Urine color change
- ☐ Vaginal dryness
- ☐ Vaginitis
- ☐ Vascular leak syndrome
- ☐ Vasculitis
- ☐ Vision Changes
- ☐ Voice changes
- ☐ Vomiting
- ☐ Watery eye
- ☐ Weight change

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*Suspected medication (generic name):____________ *Patient age:________(days, weeks, months, years) Circle one

*Seriousness Criteria

☐ Intervention to prevent incapacity
☐ Is a congenital anomaly or birth defect
☐ Is life-threatening
☐ Not serious (none of the above apply)
☐ Other medically important condition
☐ Requires initial/prolonged hospitalization
☐ Results in death
☐ Results in persistent/significant incapacity

*Patient location:

☐ Admitting Department
☐ Cardiac Catheterization Laboratory
☐ Cardiovascular / Pulmonary Services
☐ Clinic, Outpatient
☐ Dialysis Unit
☐ Emergency Department
☐ Emergency Transport Vehicle
☐ Endoscopy / GI Lab
☐ Hospice
☐ Hospital, another
☐ Intensive Care Unit, Coronary
☐ Intensive Care Unit, General
☐ Intensive Care Unit, Medical
☐ Intensive Care Unit, Neonatal
☐ Intensive Care Unit, Pediatric
☐ Intensive Care Unit, Surgical
☐ Labor / Delivery
☐ Long-Term Care Facility
☐ Maternity
☐ Nursery
☐ Nursing (Patient Care) Unit
☐ Obstetrical Recovery Room
☐ Occupational Therapy
☐ Operating Room
☐ Oncology Department
☐ Outpatient Surgery Department
☐ Patient home / Residence
☐ Pediatrics
☐ Pharmacy, Inpatient
☐ Pharmacy, Outpatient
☐ Physical Therapy
☐ Post-anesthesia Care Unit
☐ Pre-Op holding
☐ Psychiatric, Inpatient
☐ Psychiatric, Outpatient
☐ Radiology
☐ Rehabilitation Care Unit
☐ Transplant Unit

*Outcome of reaction:

☐ Fatal
☐ Not recovered/not resolved
☐ Recovered/resolved
☐ Recovered/resolved with sequelae
☐ Recovering/resolving
☐ Unknown

*Result of reaction on patient level of care:

☐ A level of care not determined
☐ Airway established / patient ventilated
☐ Antidote administered
☐ Cardiac defibrillation performed
☐ CPR administered
☐ Dialysis
☐ Drug therapy initiated / changed
☐ Hospitalization, initial
☐ Hospitalization, prolonged 1-5 days
☐ Hospitalization, prolonged 6-10 days
☐ Hospitalization, prolonged >10 days
☐ Laboratory tests performed
☐ Narcotic antagonist administered
☐ None
☐ Observation initiated / increased
☐ Oxygen administered
☐ Surgery performed
☐ Transferred to higher level of care
☐ Vital signs / monitoring initiated / increased
☐ X-ray / MRI / other diagnostic test performed

*Preventability assessment:

☐ Considered preventable, Other
☐ Documented drug interaction involved
☐ Dose/route/frequency inappropriate
☐ Drug inappropriate for clinical condition
☐ History of allergy/previous reaction
☐ Not considered preventable
☐ Poor compliance involved
☐ Required monitoring/tests not performed
☐ Unknown/Unable to assess

*Action(s) taken:

☐ Documented ADR-other
☐ Documented ADR-patient chart
☐ Documented ADR-prescription entry system
☐ Formulary changed
☐ Informed patient/caregiver
☐ Informed patient's physician
☐ Other Medication(s) Dose changed/withdrawn
☐ Policy/Procedure changed
☐ Policy/Procedure instituted
☐ Provided Supportive/Palliative Care
☐ Submitted FDA MedWatch Form
☐ Suspected Medication(s) Discontinued
☐ Suspected Medication(s) Dose Increased
☐ Suspected Medication(s) Dose Reduced
☐ Suspected Medication(s) Dose Unchanged
☐ Unknown

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***Naranjo Scale**
Probability/Causality Assessment

Question	Yes	No	Do Not Know
1. Are there previous conclusive reports on this reaction?			
2. Did the adverse event appear after the suspected drug was administered?			
3. Did the adverse reaction improve when drug was discontinued or a specific antagonist was administered?			
4. Did the adverse reaction appear when the drug was readministered?			
5. Are there alternative causes (other than the drug) that could on their own have caused the reaction?			
6. Did the reaction appear when a placebo was given?			
7. Was the drug detected in the blood (or other fluids) in concentrations known to be toxic?			
8. Was the reaction more severe when the dose was increased, or less severe when the dose was decreased?			
9. Did the patient have a similar reaction to the same or similar drugs in any previous exposure?			
10. Was the adverse event confirmed by any objective evidence?			

Brand Name: ____________
Dosage form: ____________
Batch Lot number: ____________
Strength/Concentration: ____________
Dosage Interval: ____________
Medication Start Date: ____________

Manufacturer: ____________
Intended route of administration: ____________
Investigational drug name: ____________
Indication: ____________
Dosage Information Detail: ____________
Medication End Date: ____________

Therapeutic Classification: ____________
Labeler: ____________
Compounded Ingredients: ____________
Dosage: ____________
Reaction Latency: ____________

Detection Method:

- ☐ Patient-Objective Evidence
- ☐ Triggers-Medication Orders/Lab Values
- ☐ Patient-Subjective Complaint
- ☐ Spontaneous report
- ☐ Chart Review
- ☐ Other
- ☐ ICD-9 Code Review

Patient Sex: ☐ Male ☐ Female ☐ Unavailable

Patient weight: ____________
Location detail: ____________
Duration of reaction: ____________

Pertinent medication history; medical history, including relevant pre-existing conditions; concomitant drug therapy, with dates:

Results of relevant tests/procedures to the reaction:

Reporter level of staff:

- ☐ Anesthesiologist
- ☐ Emergency Medical Services
- ☐ Nurse Anesthetist
- ☐ Nurse, Registered
- ☐ Optometrist
- ☐ Pharmacy Technician
- ☐ Physician Assistant
- ☐ Radiology Technician
- ☐ Unlicensed Assistive Personnel
- ☐ Dentist and related specialties
- ☐ Laboratory Personnel
- ☐ Nurse Practitioner/Advanced Practice Nurse
- ☐ Nurse, Travel
- ☐ Patient/Family Member/Caregiver
- ☐ Phlebotomist
- ☐ Physician, Intern
- ☐ Respiratory Therapist
- ☐ Dialysis Technician
- ☐ Management Personnel
- ☐ Nurse, Graduate
- ☐ Nursing Assistant/Aide
- ☐ Pharmacist
- ☐ Physical Therapist
- ☐ Physician, Resident
- ☐ Student
- ☐ Dietary Personnel
- ☐ Nuclear Medicine Technician
- ☐ Nurse, Licensed Practical/Vocational
- ☐ Nursing Personnel, non-specific
- ☐ Pharmacy Personnel, non-specific
- ☐ Physician
- ☐ Psych Tech/Mental Health Technician
- ☐ Unit Secretary/Clerk

Action taken and recommendations to avoid future reactions:

Internal control: ____________
Serum Creatinine: ____________ **milligrams per deciliter**

Physician Service detail: ____________
Patient detail: ____________
Reporter detail: ____________

This document is part of a quality improvement process
CONFIDENTIAL-Not to be included as part of the patient medical record

Patient Name: ____________
Medical Record #: ____________
Signature of Unit Manager: ____________

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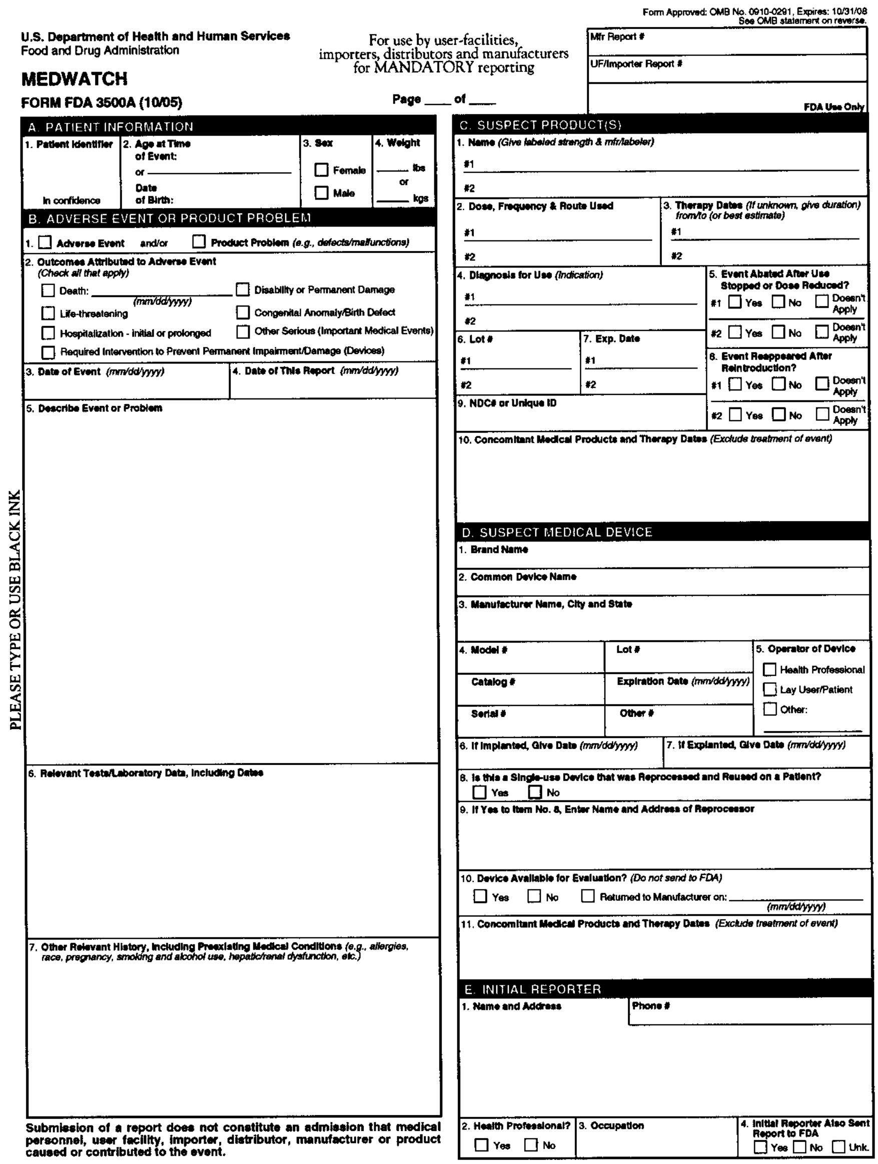

Form Approved: OMB No. 0910-0291, Expires: 10/31/08
See OMB statement on reverse.

U.S. Department of Health and Human Services
Food and Drug Administration

MEDWATCH

FORM FDA 3500A (10/05)

For use by user-facilities,
importers, distributors and manufacturers
for MANDATORY reporting

Page ____ **of** ____

Mfr Report #

UF/Importer Report #

FDA Use Only

PLEASE TYPE OR USE BLACK INK

A. PATIENT INFORMATION

1. Patient Identifier In confidence	**2. Age at Time of Event:** or ________ **Date of Birth:**	**3. Sex** ☐ **Female** ☐ **Male**	**4. Weight** ____ lbs or ____ kgs

B. ADVERSE EVENT OR PRODUCT PROBLEM

1. ☐ **Adverse Event** and/or ☐ **Product Problem** *(e.g., defects/malfunctions)*

2. **Outcomes Attributed to Adverse Event** *(Check all that apply)*
- ☐ Death: ____________ *(mm/dd/yyyy)*
- ☐ Disability or Permanent Damage
- ☐ Life-threatening
- ☐ Congenital Anomaly/Birth Defect
- ☐ Hospitalization - initial or prolonged
- ☐ Other Serious (Important Medical Events)
- ☐ Required Intervention to Prevent Permanent Impairment/Damage (Devices)

3. **Date of Event** *(mm/dd/yyyy)*

4. **Date of This Report** *(mm/dd/yyyy)*

5. **Describe Event or Problem**

6. **Relevant Tests/Laboratory Data, Including Dates**

7. **Other Relevant History, Including Preexisting Medical Conditions** *(e.g., allergies, race, pregnancy, smoking and alcohol use, hepatic/renal dysfunction, etc.)*

Submission of a report does not constitute an admission that medical personnel, user facility, importer, distributor, manufacturer or product caused or contributed to the event.

C. SUSPECT PRODUCT(S)

1. **Name** *(Give labeled strength & mfr/labeler)*
#1
#2

2. **Dose, Frequency & Route Used**
#1
#2

3. **Therapy Dates** *(If unknown, give duration) from/to (or best estimate)*
#1
#2

4. **Diagnosis for Use** *(Indication)*
#1
#2

5. **Event Abated After Use Stopped or Dose Reduced?**
#1 ☐ Yes ☐ No ☐ Doesn't Apply
#2 ☐ Yes ☐ No ☐ Doesn't Apply

6. **Lot #**
#1
#2

7. **Exp. Date**
#1
#2

8. **Event Reappeared After Reintroduction?**
#1 ☐ Yes ☐ No ☐ Doesn't Apply
#2 ☐ Yes ☐ No ☐ Doesn't Apply

9. **NDC# or Unique ID**

10. **Concomitant Medical Products and Therapy Dates** *(Exclude treatment of event)*

D. SUSPECT MEDICAL DEVICE

1. **Brand Name**

2. **Common Device Name**

3. **Manufacturer Name, City and State**

4. **Model #**
Lot #
Catalog #
Expiration Date *(mm/dd/yyyy)*
Serial #
Other #

5. **Operator of Device**
- ☐ Health Professional
- ☐ Lay User/Patient
- ☐ Other: ________

6. **If Implanted, Give Date** *(mm/dd/yyyy)*

7. **If Explanted, Give Date** *(mm/dd/yyyy)*

8. **Is this a Single-use Device that was Reprocessed and Reused on a Patient?**
☐ Yes ☐ No

9. **If Yes to Item No. 8, Enter Name and Address of Reprocessor**

10. **Device Available for Evaluation?** *(Do not send to FDA)*
☐ Yes ☐ No ☐ Returned to Manufacturer on: ____________ *(mm/dd/yyyy)*

11. **Concomitant Medical Products and Therapy Dates** *(Exclude treatment of event)*

E. INITIAL REPORTER

1. **Name and Address**

Phone #

2. **Health Professional?**
☐ Yes ☐ No

3. **Occupation**

4. **Initial Reporter Also Sent Report to FDA**
☐ Yes ☐ No ☐ Unk.

MEDWATCH

FORM FDA 3500A (10/05) *(continued)*

Page ____ **of** ____

FDA USE ONLY

F. FOR USE BY USER FACILITY/IMPORTER *(Devices Only)*

1. **Check One**
☐ User Facility ☐ Importer

2. **UF/Importer Report Number**

3. **User Facility or Importer Name/Address**

4. **Contact Person**

5. **Phone Number**

6. **Date User Facility or Importer Became Aware of Event** *(mm/dd/yyyy)*

7. **Type of Report**
☐ Initial
☐ Follow-up # ________

8. **Date of This Report** *(mm/dd/yyyy)*

9. **Approximate Age of Device**

10. **Event Problem Codes** *(Refer to coding manual)*
Patient Code ☐ - ☐ - ☐
Device Code ☐ - ☐ - ☐

11. **Report Sent to FDA?**
☐ Yes ________ *(mm/dd/yyyy)*
☐ No

12. **Location Where Event Occurred**
☐ Hospital
☐ Home
☐ Nursing Home
☐ Outpatient Treatment Facility
☐ Outpatient Diagnostic Facility
☐ Ambulatory Surgical Facility
☐ Other: ________ *(Specify)*

13. **Report Sent to Manufacturer?**
☐ Yes ________ *(mm/dd/yyyy)*
☐ No

14. **Manufacturer Name/Address**

G. ALL MANUFACTURERS

1. **Contact Office - Name/Address** ***(and Manufacturing Site for Devices)***

2. **Phone Number**

3. **Report Source** *(Check all that apply)*
☐ Foreign
☐ Study
☐ Literature
☐ Consumer
☐ Health Professional
☐ User Facility
☐ Company Representative
☐ Distributor
☐ Other:
____
____
____
____

4. **Date Received by Manufacturer** *(mm/dd/yyyy)*

5.
(A)NDA # ________
IND # ________
STN # ________
PMA/ 510(k) # ________
Combination Product ☐ Yes
Pre-1938 ☐ Yes
OTC Product ☐ Yes

6. **If IND, Give Protocol #**

7. **Type of Report** *(Check all that apply)*
☐ 5-day ☐ 30-day
☐ 7-day ☐ Periodic
☐ 10-day ☐ Initial
☐ 15-day ☐ Follow-up # ____

8. **Adverse Event Term(s)**

9. **Manufacturer Report Number**

The public reporting burden for this collection of information has been estimated to average 66 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

H. DEVICE MANUFACTURERS ONLY

1. **Type of Reportable Event**
☐ Death
☐ Serious Injury
☐ Malfunction
☐ Other: ________

2. **If Follow-up, What Type?**
☐ Correction
☐ Additional Information
☐ Response to FDA Request
☐ Device Evaluation

3. **Device Evaluated by Manufacturer?**
☐ Not Returned to Manufacturer
☐ Yes ☐ Evaluation Summary Attached
☐ No *(Attach page to explain why not)* or provide code:
____

4. **Device Manufacture Date** *(mm/yyyy)*

5. **Labeled for Single Use?**
☐ Yes ☐ No

6. **Evaluation Codes** *(Refer to coding manual)*
Method ☐ - ☐ - ☐ - ☐
Results ☐ - ☐ - ☐ - ☐
Conclusions ☐ - ☐ - ☐ - ☐

7. **If Remedial Action Initiated, Check Type**
☐ Recall ☐ Notification
☐ Repair ☐ Inspection
☐ Replace ☐ Patient Monitoring
☐ Relabeling ☐ Modification/ Adjustment
☐ Other: ________
____

8. **Usage of Device**
☐ Initial Use of Device
☐ Reuse
☐ Unknown

9. **If action reported to FDA under 21 USC 360i(f), list correction/ removal reporting number:**

10. ☐ **Additional Manufacturer Narrative** and / or 11. ☐ **Corrected Data**

Department of Health and Human Services
Food and Drug Administration - MedWatch
10903 New Hampshire Avenue
Building 22, Mail Stop 4447
Silver Spring, MD 20993-0002
Please DO NOT RETURN this form to this address.

OMB Statement:
"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number."

Appendix F

Biographical Sketches of Workshop Speakers

LT. COL. Sonya Corum, M.B.A., is currently serving as Director of the Experimentation and Analysis Element at the U.S. Army Training Center, Fort Jackson, South Carolina. She graduated with a B.S. in Food and Nutrition from Appalachian State University in Boone, North Carolina, in 1988. Her first assignment after completing the Dietetic Internship at Brooke Army Medical Center was at Ireland Army Community Hospital, Fort Knox, Kentucky, as the Chief, Clinical Dietetics. Since that time, Lieutenant Colonel Corum has held a number of positions in medical treatment facilities including Chief, Production and Service at William Beaumont Army Medical Center, and Chief, Nutrition Care at Fort Campbell. She also deployed with the 86th Combat Support Hospital in support of Hurricane Mitch relief. She has served as the Executive Fellow to the Chief, Medical Specialist Corps. Lieutenant Colonel Corum was the Nutrition Staff Officer at the U.S. Army Center for Health Promotion and Preventive Medicine (USA-CHPPM) where she spearheaded the Army's Dietary Supplement Education Campaign. She also deployed with the 31st Combat Support Hospital in support of Operation Iraqi Freedom. Lieutenant Colonel Corum received an M.B.A. from the University of Texas, El Paso, and is a Command and General Staff College graduate.

Gerald J. Dal Pan, M.D., M.H.S., is the Director of the Office of Surveillance and Epidemiology in the U.S. Food and Drug Administration's Center for Drug Evaluation and Research (FDA/CDER). Prior to that, he was the Director of the Division of Surveillance, Research, and Communication Support in CDER's Office of Drug Safety, a position he had held since

December 2003. He received his medical degree from Columbia University, and his master's degree in clinical epidemiology from Johns Hopkins University. He trained in internal medicine at the Hospital of the University of Pennsylvania, and in neurology at the Johns Hopkins Hospital. He is board certified in internal medicine and neurology. Dr. Dal Pan was an instructor in the Neurology Department at Johns Hopkins University. He next worked for Guilford Pharmaceuticals in Baltimore, and then for HHI Clinical Research and Statistical Services in Hunt Valley, Maryland. He joined the FDA in July 2000 as a medical officer in the Division of Anesthetic, Critical Care, and Addiction Drug Products.

Vasilios Frankos, Ph.D., serves as the Director, Division of Dietary Supplement Programs (DDSP) and the lead scientist for dietary supplements for the FDA and is responsible for the full implementation of the Dietary Supplement Health and Education Act of 1994. He directs and coordinates policy and administrative activities within the division. He advises on policy and management issues on dietary supplement programs, new dietary ingredient safety assessments, good manufacturing practice, and adverse reaction monitoring, and related activities pertaining to dietary supplements. Before becoming the Director of DDSP, Dr. Frankos also served as Special Assistant for Dietary Supplement Science Review providing toxicological and pharmacological evaluation of data used to assess the risks posed by dietary supplement products, Staff Science Advisor in the Office of the Commissioner, and as a Senior Toxicologist in the Center for Food Safety and Applied Nutrition. Dr. Frankos received his M.S. in molecular biology from the University of Maryland and his Ph.D. in pharmacology and toxicology from the University of Maryland Pharmacy School. He has over 30 years' experience in the toxicological and pharmacological evaluation of data used to assess the safety of FDA-regulated products. In addition to his FDA activities, he spent 18 years as a principal in the consulting firm ENVIRON International Corp.

Steve French, M.B.A., is the Managing Partner of the Natural Marketing Institute, with over 25 years of strategic marketing, business development, market research, and management experience. Complementing a B.S. and M.B.A. in marketing, Mr. French has accumulated extensive insight and knowledge into health, wellness, environmentalism, and social responsibility. He has unparalleled experience across a wide range of corporate business functions and has pioneered a range of consumer research databases. Mr. French is a frequent speaker at many industry events and conferences, and is regularly utilized by television, radio, magazine, newspaper, Internet, and other media sources. He is also an author of numerous published articles and written research reports used across many industries.

Paula Gardiner, M.D., M.P.H., is an Assistant Professor in the Department of Family Medicine at Boston University Medical School. She is a former Research Fellow at the Division for Research and Education in Complementary and Integrative Medical Therapies, Osher Institute, Harvard Medical School. Her research focuses on use of dietary supplements by adults and adolescents in the U.S. population. Additionally, her research has focused on the safety issues surrounding dietary supplements, such as prescription medication and dietary supplement interactions and adverse event reporting. As a member of the United States Pharmacopeia's Dietary Supplements Expert Committee, she has focused on improving adverse event reporting in the United States.

Mary Hardy, M.D., serves as the Codirector of the Integrative Medicine Health and Wellness Program at the Venice Family Clinic, the largest free clinic in the United States. Her clinical practice now involves educating cancer patients in integrative therapies at the Ted Mann Family Integrative Oncology Program at the University of California, Los Angeles. Her current research interests include reviewing the evidence for the safety and efficacy of natural therapies, especially botanicals. Dr. Hardy is board certified in internal medicine and a specialist in botanical and integrative medicine and has actively combined complementary and alternative therapies with traditional Western medicine for many years. A graduate of Louisiana State University School of Medicine in New Orleans, Dr. Hardy completed her internal medicine residency at the Tufts New England Medical Center before studying medical ethics at Harvard Divinity School and Loma Linda University. She completed advanced training in botanical medicine at the Institute for Medical Herbalism and has studied with practitioners in Peru, Kenya, South Africa, and China. She is the complementary and alternative medicine expert for a number of research projects conducted by the Southern California Evidence-Based Practice Center of the RAND Corporation. In addition, she has expanded her interest in botanical research by serving for two and a half years as the Associate Director of the Botanical Research Center of the University of California, Los Angeles, funded by the National Institutes of Health (NIH). Dr. Hardy also serves on the scientific advisory board of the American Botanical Council and the editorial boards of *Alternative Medicine Alert*, *Alternative Therapies in Women's Health*, *Evidence-Based Complementary and Alternative Medicine*, *FACT* as well as *Phytomedicine*. Dr. Hardy is recognized as an authority on integrative medicine and natural products by organizations such as the NIH Office of Dietary Supplements, the California Medical Board, the American Medical Association, the American Pharmaceutical Association, CBS, NBC, Discovery Channel, and the *Los Angeles Times*. She is a founding member of the Advisory Council for the newly established Naturopathic Medicine Board of California and has recently been appointed to the External Advisory Council for the Natural Product Directorate of the Canadian government. The multidisciplinary clinic she founded at Cedars-Sinai Medical Center in the department of Medicine in 1998 allowed her to explore the practical and philosophical issues that both facilitate and impede the development of integrative medicine as a discipline. Contributing to the national development of integrative medicine, she serves as the cochairperson of the Clinical Practice Committee of the Academic Consortium of Integrative Medicine (an organization of the leading medical schools practicing and teaching in this area). Dr. Hardy recently completed a book for Reader's Digest, *Best Remedies*, which focuses on integrative medicine. She is also conducting a review of the quality of research trials in herbal medicine and is finishing a systematic review on the effects of dietary supplements on coagulation for the NIH Office of Dietary Supplements.

LT. COL. Paul J. Hoerner is currently the Deputy Director of the U.S. Department of Defense (DoD) Patient Safety Center (PSC). He directs the daily operations of the PSC, with a budget of $1.5 million, serving more than 576 DoD medical treatment facilities and 9.2 million beneficiaries worldwide. This includes quarterly, annual, and bimonthly publications, safety alerts, and focused analysis for use by DoD, Army, Navy, and Air Force facilities to improve patient safety. Arriving at the PSC in September 2006, he had recently completed a 1-year fellowship at a medial malpractice insurance company. Prior to that assignment, he was the Chief of Pharmacy Operations at David Grant Medical Center. The pharmacy processed over 3,500 outpatient prescriptions and prepared over 1,600 inpatient orders daily. He provided oversight of all aspects of the pharmacy's various elements (clinical, support, inpatient, and outpatient). Colonel Hoerner's career goals are to advance his leadership capabilities and expertise in the fields of pharmacy and patient safety.

LT. COL. Danny B. N. Jaghab has been the Nutrition Staff Officer for the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) at Aberdeen Proving Ground, Maryland, since 2004. His work includes analyzing and assessing current policies and developing and implementing new health promotion and nutrition policies and regulations that better meet the needs of the DoD. He collaborates with federal and national agencies on projects and initiatives as the sole subject-matter expert on nutrition for the U.S. Army. From July 2002 to July 2004, he directed the U.S. Military Dietetic Internship Consortium at the Brooke Army Medical Center in San Antonio, Texas. He was also previously the Chief of the Nutrition Care Division at the DeWitt Army Community Hospital in Fort Belvoir, Virginia. He was a Bronze Star Medal recipient during Operation

Desert Shield/Storm; recipient of the 2004 American Dietetic Association's Media Excellence Award; recipient of the 2004 Department of Defense Patient Safety Award in the category of Technology; and was elected the 2006 Career Guidance Chair, American Dietetic Association's National Organization of Men in Nutrition. He received his B.S. in nutrition and dietetics from Drexel University in Philadelphia, Pennsylvania, and his M.S. in education and counseling from the Long Island University in West Point, New York.

David Kaufman, Sc.D., is Professor of Epidemiology at the Boston University School of Public Health. He obtained his B.A. in 1973 from Bethel College, North Newton, Kansas, and his M.S. in 1979 and Sc.D. in 1983 in Epidemiology from the Harvard School of Public Health. In 1975, he joined the newly created Drug Epidemiology Unit (DEU) (subsequently renamed Slone Epidemiology Unit, and since 2001 the Slone Epidemiology Center) as a Research Associate. Dr. Kaufman was Assistant Director of the Slone Epidemiology Unit from 1986 to 1997, and has been Associate Director since 1998. His early career as an epidemiologist at the DEU was primarily spent in studies of drugs and other factors in relation to cancer, heart disease, and various other conditions. Together with Drs. Slone, Shapiro, and Lynn Rosenberg, he participated in the development of Case-Control Surveillance. In the 1980s, Dr. Kaufman was coinvestigator of the International Agranulocytosis and Aplastic Anemia Study, a pioneering effort in the evaluation of these extremely rare but often drug-induced blood dyscrasias, that was conducted in seven countries with several hundred cases enrolled; he coauthored a book describing the results. He has since directed studies of aplastic anemia in Thailand and the United States and served as an adviser to a study of that disease in Brazil. The Thai study is the largest epidemiological investigation of aplastic anemia that has been conducted, with over 500 cases and 2,200 controls. Dr. Kaufman pursued his interest in rare drug-induced diseases as principal investigator of an international study of Stevens-Johnson syndrome and toxic epidermal necrolysis conducted in four countries in Europe, and a study of anaphylaxis conducted in Spain, Hungary, India, and Sweden. Other major activities have included an international study of analgesics in relation to upper gastrointestinal bleeding, and more recently, the National Analgesic Nephropathy Study, a multicenter study of end-stage renal disease patients in three regions of the United States. Dr. Kaufman worked closely with Allen Mitchell in the development of the Slone Survey, a U.S. population-based survey of medication and dietary supplement use that was initiated in 1998; he is principally responsible for the implementation of that project. Another current study is exploring the relationship of *Oxalobacter formigenes*, an oxalate-metabolizing bacterium found in the colons of about 70 percent of the normal population, and calcium oxalate kidney stones. Dr. Kaufman is also the Principal Investigator of recently launched national patient registries of multiple myeloma and myelodysplastic syndromes (Patient Registries at Slone: Myeloma and MDS).

Rick Kingston, PharmD., is President, Regulatory and Scientific Affairs for SafetyCall International, a multidisciplinary medical practice and poison center focused on corporate postmarket surveillance and product safety for drugs, dietary supplements, and consumer products. He has a 28-year academic career, having attained the rank of full professor in the Department of Experimental and Clinical Pharmacology, and is currently serving as Clinical Professor of Pharmacy in the College of Pharmacy at the University of Minnesota. At the University of Minnesota, he is course director for the didactic course "Therapeutics of Herbs and Other Natural Medicinals" taught in the College of Pharmacy and has previously served as a member of the University's Center for Plants and Human Health and the National Center for Complementary and Alternative Medicine-funded integrative medicine Center for Spirituality and Healing. Previously, Dr. Kingston was cofounder and former Director of the Minnesota Regional Poison Center and its affiliated industry toxicology and product safety surveillance service programs at St. Paul-Ramsey Medical Center in St. Paul, Minnesota. Dr. Kingston completed his B.S. in Pharmacy at the University of New Mexico, his PharmD. in Clinical Pharmacy at the University of Minnesota, and a postdoctoral fellowship in clinical toxicology and pharmacokinetics at St. Paul-Ramsey Medical Center and the University of Minnesota. His 28-year professional experience has been centered in the areas of clinical toxicology and pharmacology, poison control, product postmarket surveillance, and drug and dietary supplement safety. His research and practice interests coincide with SafetyCall International initiatives for corporate clients and include a focus on injury prevention, the epidemiology of toxicology-related incident data, product safety and related regulatory affairs, and specific advances in the clinical management or triage of patients being treated or evaluated for drug, toxin, or consumer product exposures.

Harris R. Lieberman, Ph.D., is a Research Psychologist in the Military Nutrition Division of the U.S. Army Research Institute of Environmental Medicine (USARIEM) in Natick, Massachusetts. He is an internationally recognized expert in the area of nutrition and behavior and has published over 125 original, full-length papers in scientific journals and edited books. Dr. Lieberman received his Ph.D. in Physiological Psychology in 1977 from the University of Florida and then conducted postdoctoral research at the Department of Psychology and Brain Science at the Massachusetts Institute of Technology (MIT). From 1980 to 1990 he was on the staff at MIT, where he examined the effects of food constituents and drugs on human behavior and brain function. In 1990 he joined the civilian research staff of USARIEM where he continued his work in nutrition, behavior, and stress. From 1994 to 2000 he was Chief or Deputy Chief of the Military Nutrition program at USARIEM. His recent research has addressed the effects of various nutritional factors, diets, and environmental stress on cognitive performance and brain function. He holds two patents for novel technologies to assess and enhance cognitive performance. He currently chairs his institute's scientific research committee and serves on the DoD Dietary Supplements Committee and several other national and international committees.

Bernadette M. Marriott, Ph.D., is Principal Associate and Senior Scientist at Abt Associates, and has 38 years of experience in the fields of nutrition, psychology, and comparative medicine, with expertise in diet, nutrition and chronic disease. Dr. Marriott has worked in scientific settings in the federal government, universities, and foundations. Her research has focused on nutrition and related behavioral studies, specifically diet and health research, and food labeling. She currently holds an adjunct full professor position in the Department of Nutritional Sciences, University of North Carolina School of Medicine, and has held previous academic positions of assistant through full professor. Other positions include Vice President for Science Integration, RTI International, Founding Director of the NIH Office of Dietary Supplements, Deputy Director of the Food and Nutrition Board of the Institute of Medicine (IOM), Vice Provost for Research and Dean of Graduate Studies at Northern Arizona University, Vice President for Programs and Communications for the Burroughs Wellcome Fund, and Associate Director of the Caribbean Primate Research Center, University of Puerto Rico. While at the IOM, she oversaw the activities of the Committee on Military Nutrition Research for 8 years and is very familiar with the nutrient requirements of active duty personnel, nutrient composition of ration packages, exercise, and weight management in the military services. At the IOM, she edited, helped write, and/or oversaw the production of 24 reports; of particular relevance to this study are *Food Components to Enhance Performance (DoD)* and *Guiding Principles for Nutrition Labeling and Food Fortification*, for which she was a special consultant and which addresses foods and dietary supplements. Dr. Marriott has extensive experience managing complex programs, building consensus, and working with scientific data. She currently is principal investigator on two NIH-funded projects on adult and child intake, a corporate-funded project on infant feeding, an obesity study supported by the Office of Science and Technology Policy, and was the scientific lead for diet, exercise, weight management, and related health indicators on the *2005 Department of Defense (DoD) Survey of Health Related Behaviors Among Military Personnel* with Bob Bray. Prior to leaving RTI, she was principal investigator on the TRICARE Management Activity-funded *Weight Management Demonstration Project:*

Healthy Eating and Active Living in TRICARE Households (HEALTH). Bernadette Marriott has a B.Sc. in biology/immunology from Bucknell University, a Ph.D. in psychology from King's College, Aberdeen, Scotland, and postgraduate training in trace mineral nutrition, comparative medicine, and advanced statistics. She has published extensively, is on a number of national committees, and is a frequent speaker on diet, dietary supplements, and health.

Scott J. Montain, Ph.D., is a research physiologist working in the Military Nutrition Division at USARIEM, Natick, Massachusetts. Dr. Montain received an M.S. from Ball State University in 1984 and a Ph.D. from the University of Texas at Austin in 1991 before completing postdoctoral training at USARIEM. He is author or coauthor of over 95 peer-reviewed journal articles, book chapters, and reports. Dr. Montain currently serves as Councilor for the Exercise and Environmental Physiology section of the American Physiological Society, and is a Fellow of the American College of Sports Medicine.

LT. COL. Charity Thomasos, M.S., is currently stationed at Eglin Air Force Base, Florida, as the Director of Nutritional Medicine Flight. She earned her B.S. in clinical nutrition/dietetics from West Virginia Wesleyan College, Buckhannon, West Virginia, and her M.S. in human nutrition/biochemistry from the Ohio State University, Columbus. She is a member of the American Dietetic Association and the Nutrition and Complementary Medicine practice group. Lieutenant Colonel Thomasos is the Air Force Dietetics Representative to the DoD Dietary Supplement Committee and a member of the Special Operations Forces subcommittee.

Andrew J. Young, Ph.D., is a research physiologist and Chief of the Military Nutrition Division at USARIEM in Natick, Massachusetts. He obtained a B.S. in biology at Virginia Military Institute and a Ph.D. in physiology at North Carolina State University, then served in the U.S. Army with assignments at USARIEM (1977–1981) and at the Walter Reed Army Institute of Research (1981–1983). After leaving the Army, Dr. Young continued as a civilian scientist at USARIEM. His research has concerned the biological basis for, and strategies to mitigate, performance degradation in people experiencing intense physical exertion, sleep restriction, nutritional deprivation, and exposure to extremes of heat, cold, and high altitude, all of which are characteristics of sustained combat operations. Dr. Young is a member of the American Physiological Society and a Fellow of the American College of Sports Medicine. He is also Editor in Chief of the American College of Sports Medicine's flagship scientific journal, *Medicine and Science in Sports and Exercise.*

Appendix G

Biographical Sketches of Committee Members

M.R.C. Greenwood, Ph.D., is Professor of Nutrition and Internal Medicine, Chair of the graduate group in Nutritional Biology, Director of the Foods for Health Initiative at the University of California, Davis, and Chancellor Emerita, University of California, Santa Cruz. Her previous positions include Chancellor of the University of California, Santa Cruz, from 1996 to 2004 and University of California Provost and Senior Vice President of Academic Affairs. Prior to her Santa Cruz appointments, she was Dean of Graduate Studies and Vice Provost for Academic Outreach and Professor of Biology and Internal Medicine at University of California, Davis. Prior to that, she taught at Vassar College, where she was Chair of the Department of Biology and Director of the Undergraduate Research Summer Institute and was on the adjunct faculty of Columbia University's medical school. From November 1993 to May 1995, while on leave from the University of California, Davis, Greenwood served as Associate Director for Science at the Office of Science and Technology Policy in the Executive Office of the President of the United States. She has been a member of the Institute of Medicine (IOM) of the National Academies (NAS) since 1992. She is currently the Chair of the NAS Policy and Global Affairs Committee. She was a member of the Food and Nutrition Board from 1985 to 1990 and served as chair from 1990 to 1993. She is also a Fellow of the American Academy of Arts and Sciences as well as the American Association for the Advancement of Science, serving as its president in 1998 and chair in 1999. She has been President of the American Society for Clinical Nutrition and NAASO, the Obesity Society. Dr. Greenwood received her A.B., summa cum laude, from Vassar College, and received her Ph.D. from Rockefeller University. Her research areas include obesity, diabetes, and women's health.

Cheryl Anderson, Ph.D., M.P.H., is an Assistant Professor in the Department of Epidemiology at the Johns Hopkins Bloomberg School of Public Health. Previously, she was an Instructor of Epidemiology at the University of Pennsylvania School of Medicine, Center for Clinical Epidemiology and Biostatistics. Dr. Anderson's research centers on diet and the prevention of chronic diseases in minority and underserved populations. Her current research projects address diet and the prevention of cardiovascular disease (CVD) in the context of chronic kidney disease and the optimal macronutrient intake in CVD prevention. Dr. Anderson is a member of the American Heart Association Committee on Nutrition and Physical Activity. She is also a Dannon Institute Nutrition Leadership Institute (NLI) Scholar and the current President of the NLI Alumni Association. She recently served on the National Institutes of Health (NIH) National Institute of Neurologic Disorders and Stroke Writing Group on Primary Prevention of Stroke. She has an A.B. from Brown University, M.P.H. from the University of North Carolina at Chapel Hill, and an M.S. in epidemiology and a Ph.D. in nutritional sciences from the Department of Epidemiology at the University of Washington School of Public Health and Community Medicine.

Bruce R. Bistrian, M.D., Ph.D., is Professor of Medicine at Harvard Medical School and Chief of Clinical Nutrition, Beth Israel Deaconess Medical Center. Formerly he was Codirector of Hyperalimentation Services, New England Deaconess Hospital, and a lecturer in the Department of Nutrition and Food Science, Massachusetts Institute of Technology (MIT). Dr. Bistrian's primary research interests include nutritional assessment, the metabolic effects of acute infections, nutritional support of hospitalized patients, and the pathophysiology of protein-calorie malnutrition. He is a Fellow of the American College of Physicians, and has received an honorary M.A. from Harvard University. Dr. Bistrian is the 2004 recipient of the Goldberger Award of the American Medical Association. Dr. Bistrian has been President of the American Society for Parenteral and Enteral Nutrition, President of the American Society of Clinical Nutrition, and President of the Federation of American Societies of Experimental Biology. He currently serves on the IOM Committee on Military Nutrition Research. Dr. Bistrian has served on the editorial boards of numerous nutrition and medical journals, and is the author or coauthor of over 400 articles for scientific publications. He earned his M.D. from Cornell University, his M.P.H. from Johns Hopkins University, and his Ph.D. in nutritional biochemistry and metabolism from MIT. Dr. Bistrian is board certified in internal medicine and was certified in critical care medicine from 1987 to 2007.

John W. Erdman, Jr., Ph.D., is Professor of Nutrition and Food Science in the Department of Food Science and Human Nutrition and a Professor in the Department of Internal Medicine at the University of Illinois at Urbana-Champaign. His research interests include the effects of food processing on nutrient retention, the metabolic roles of vitamin A and beta-carotene, and the effects of tomatoes and broccoli and their bioactive components on risk of prostate cancer. His research regarding soy protein has extended into studies on the impact of nonnutrient components of foods, such as phytoestrogens, on chronic disease. Dr. Erdman has published over 160 peer-reviewed research papers. He chaired the 1988 Gordon Conference on Carotenoids, and has served as a Burroughs Wellcome Visiting Professor in Basic Medical Sciences at the University of Georgia, and the G. Malcolm Trout Visiting Scholar at Michigan State University. His awards include the Borden Award from the American Society for Nutritional Sciences and the Babcock-Hart Award from the Institute of Food Technologists. Dr. Erdman has served on many editorial boards, and on many program and planning committees for the American Society of Nutritional Sciences, the Institute of Food Technologists, and the NAS. In 1992, he was elected a Fellow of the Institute of Food Technologists, and in 2003 he was elected a Fellow of the American Heart Association. Dr. Erdman was elected to the IOM in 2003. He received his M.S. and Ph.D. in food science from Rutgers University.

William C. Franke, Ph.D., is an Associate Director of the Center for Advanced Food Technology at Rutgers University. He provides technical expertise in the area of product development and food regulations, especially as related to functional foods and nutraceuticals, and develops new opportunities for technology transfer to small and large companies. He also contributes to administration, marketing, and strategic planning. He is a coprincipal investigator for a U.S. Department of Defense contract to develop combat rations to improve the physical and mental performance of soldiers under stress. Previously, he spent 28 years at Lipton/Unilever and served in a number of senior management positions in product development, quality assurance, and regulatory affairs. Most recently, he was Vice President for Scientific and Regulatory Affairs with Unilever United States before he retired. He is a former member of the NAS/IOM Food Forum and served on the IOM expert Committee on Evaluation of the Addition of Ingredients New to Infant Formula. He also served as a member of the board of the Cancer Institute of New Jersey and was recently elected a Fellow of the Institute of Food Technologists. Dr. Franke founded Heart Blend Foods LLC, an R&D company that specializes in the development of heart-healthy foods, in 2005. The company's objective is to license technology and/or bring products to market that improve public health.

Elizabeth Jeffery, Ph.D., is Professor of Nutritional Toxicology and Professor of Nutritional Sciences at the University of Illinois. Dr. Jeffery teaches and performs research in the area of safety and efficacy of functional foods and dietary supplements, with emphasis on biochemical mechanisms of cancer prevention by broccoli and related crucifers. A toxicologist by training, Dr. Jeffery has held elected positions on the Education and Nominating Committees of the Society of Toxicology. She is past President of the Midwest Regional Chapter of the Society of Toxicology and past Chair of the Toxicology specialty section in the American Society for Pharmacology and Experimental Therapeutics. She is past Director of Research Interest Sections and past Chair of the Dietary Bioactive Components Research Interest Section of the American Nutrition Society. She served on the NAS Committee on the Framework for Evaluating the Safety of Dietary Supplements. She has served as an Associate Editor for the *Journal of Toxicology and Applied Pharmacology* and as guest Field Editor for the *Journal of Pharmacology and Experimental Therapeutics*, and is involved in the review of research grant proposals for both the NIH and the U.S. Department of Agriculture (USDA). Dr. Jeffery has a Ph.D. in biochemistry from the University of London, England.

Robin B. Kanarek, Ph.D., is Professor of Psychology at Tufts University in Medford, Massachusetts. Her prior positions include Research Fellow, Division of Endocrinology, University of California, Los Angeles (UCLA) School of Medicine; Research Fellow in Nutrition at Harvard University; and Professor of Nutrition, Chair of the Psychology Department and Dean of the Graduate School of Arts and Sciences at Tufts University. She is a member of the editorial boards of *Physiology and Behavior, Nutritional Neuroscience*, and the *Tufts Diet and Nutrition Newsletter* and is a past Editor in Chief of *Nutrition and Behavior*. Dr. Kanarek has served on ad hoc review committees for the National Science Foundation, the NIH, and USDA nutrition research, as well as the Member Program Committee of the Eastern Psychological Association. She is a fellow of the American College of Nutrition and the North American Society for the Study of Obesity; her other professional memberships include the American Institute of Nutrition, New York Academy of Sciences, Society for the Study of Ingestive Behavior, and Society for Neurosciences. Dr. Kanarek received a B.A. in biology from Antioch College in Yellow Springs, Ohio, and an M.S. and a Ph.D. in psychology from Rutgers University in New Brunswick, New Jersey.

Carl L. Keen, Ph.D., is Chairman of the Department of Nutrition at the University of California, Davis, and a Professor of Nutrition and Internal Medicine. Dr. Keen's research focuses on the influence of diet on embryonic and fetal development, the study of gene–nutrient interactions (emphasizing how subtle changes in cell mineral concentrations influence the expression of select genes), dietary influences on oxidant defense systems and as a consequence the occurrence of cellular oxidative damage, and the influence of dietary flavonoids on vascular health. He has served on numerous government boards including California's Scientific Advisory Board for the Office of Environmental Health Hazard Assessment, numerous Environmental Protection Agency Environmental Health Grant Review Panels, the USDA Human Nutrient Requirements Study Section, and several NIH study sections. He has served on a number of editorial boards and is a member of the American Society for Nutrition, the Teratology Society, the Society for Experimental Biology and Medicine, and the American Association for the Advancement of Science.

Gail B. Mahady, Ph.D., is Associate Professor of Pharmacy Practice and Director of the Clinical Pharmacognosy Laboratory at the University of Illinois at Chicago. Dr. Mahady's research interests include the discovery of novel natural agents for treatment or prevention of infectious disease, specifically plant-based antimicrobial drugs for the prevention and treatment of *Helicobacter pylori* and *Chlamydia* infections in humans; the assessment of clinical safety and efficacy of herbal supplements globally for the symptomatic treatment of menopause, premenstrual syndrome, and chronic urinary tract infections; and the assessment of quality, safety, and efficacy of botanicals for the World Health Organization's (WHO) Traditional Medicines Programme. She is a member of research faculty within the Pan American Health Organization/WHO Collaborating Center for Traditional Medicine at the College of Pharmacy, University of Illinois at Chicago. She is an Elected Member of the United States Pharmacopeia Dietary Supplements Information Expert Panel and a member of the review panel for the National Center for Complementary and Alternative Medicine's Annual Bibliography of Significant Advances in Dietary Supplement Research, among many NIH review panels. Dr. Mahady received her Ph.D. in Pharmacognosy from the University of Illinois at Chicago.

Sanford A. Miller, Ph.D., is a Senior Fellow at the Center for Food and Nutrition Policy at the University of Maryland, College Park. In December 2000, he was named Professor and Dean Emeritus of the Graduate School of Biomedical Sciences at the University of Texas Health Science Center at San Antonio, where he was the Dean of the Graduate School of Biomedical Sciences, and Professor in the Departments of Biochemistry and Medicine from 1987 to 2000. He is the former Director of the Center for Food Safety and Applied Nutrition at the U.S. Food and Drug Administration. Previously, he was a Professor of Nutritional Biochemistry at MIT. Dr. Miller has served on many national and international government and professional society advisory committees, including as Chair of the Joint FAO/WHO

(Food and Agriculture Organization/World Health Organization) Expert Consultation on the Application of Risk Analysis to Food Standards Issues. Dr. Miller's professional honors include the Atwater Memorial Lectureship Award from the USDA Agricultural Research Service, the Babcock-Hart Award from International Life Sciences Institute and the Institute of Food Technologists, the Conrad A. Elvehjem Award of the American Institute of Nutrition, the Esther Peterson Consumer Service Award from the Food Marketing Institute, the Sterling B. Hendricks Award from the USDA, and election as Fellow of the American Society for Nutrition. In June 2000, he was the recipient of the FDA's Distinguished Alumni Award. He has been a member of many NAS committees, including the Food and Nutrition Board's Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, and Subcommittee on Upper Reference Levels of Nutrients and Panel on Macronutrients. He was named a National Associate of NAS in 2002. He is author or coauthor of more than 200 original scientific publications. Dr. Miller received a B.S. in chemistry from the City College of New York, and an M.S. and a Ph.D. in physiology and biochemistry from Rutgers University.

Esther F. Myers, Ph.D., R.D., is Director of Research and Scientific Affairs at the American Dietetic Association (ADA), a position she has held since October 2000, after retiring from the Air Force and serving as Chief Consultant to the Air Force Surgeon General. Dr. Myers currently focuses on research activities needed for the dietetics profession and the association, as well as the ADA strategic leadership initiative on obesity and the ADA Foundation initiative Healthy Weight for Kids. She has been actively involved in the development of ADA's evidence analysis process and in research projects focusing on evaluating the impact of nutrition services in Medicare Demonstration projects and in collaboration with Blue Cross and Blue Shield of North Carolina. She is the ADA staff liaison with the Nutrition Care Process and Standardized Language Committee, which is developing and validating terminology to reflect nutrition care for standardized language systems and electronic health records. Prior to joining ADA, she served as a site visitor for the Commission on Accreditation for Dietetics Education, a peer reviewer for the *Journal of the American Dietetic Association*, and a member of the Health Services Research Task Force overseeing dietetic outcomes research. She was a member of the IOM Committee on Nutrition Services for Medicare Beneficiaries. Dr. Myers received her undergraduate degree from North Dakota State University, her master's degree from the Ohio State University, and her doctorate from Kansas State University.

Janet Walberg Rankin, Ph.D., is a Professor in the Department of Human Nutrition, Foods, and Exercise at Virginia Polytechnic Institute and State

University. She has also served as Chair of its Food, Nutrition, and Health Initiative and acting Department Head as well as Associate Director, and then Interim Director of the University's new Institute for Biomedical and Public Health Sciences. Dr. Rankin's research is related to sports nutrition and interventions for obesity, with specific areas of interest that include the effects of dietary macronutrient mix, energy balance, and dietary supplements on performance, body composition, and immunity. Her current research focuses on dietary manipulations that may affect inflammation and oxidative stress in athletes and obese individuals. Dr. Rankin's affiliation with professional organizations has included membership on the Executive Board and later serving as President of the Southeast Chapter of the American College of Sports Medicine. She is a Fellow of the American College of Sports Medicine, has served as its Vice President, and has been a member or chair on many national committees for this organization. Dr. Rankin received her bachelor's degree in zoology from Duke University and her doctorate in nutrition with a minor in exercise physiology from the University of California, Davis.

Consultant

David F. Dinges, Ph.D., is Professor and Chief of the Division of Sleep and Chronobiology, and Director of the Unit for Experimental Psychiatry in the Department of Psychiatry at the University of Pennsylvania School of Medicine. His research focuses on physiological, neurobehavioral, and psychological effects of sleep loss, disturbances of circadian biology, and stress, and the implications of these unmitigated effects on health and safety. He has performed extensive scientific work on development and validation of behavioral, technological, and pharmacological countermeasures for these effects. His research has been supported by grants from the NIH, the National Aeronautics and Space Administration (NASA), the U.S. Department of Defense, the U.S. Department of Transportation, and the Department of Homeland Security. He currently leads the Neurobehavioral and Psychosocial Factors Team for the NASA-funded National Space Biomedical Research Institute. He is a member of the National Advisory Council for Nursing Research of the NIH. He has been President of the U.S. Sleep Research Society and of the World Federation of Sleep Research and Sleep Medicine Societies, and served on the Board of Directors of the American Academy of Sleep Medicine and the National Sleep Foundation. He is currently Editor in Chief of *SLEEP*. He has received numerous awards, including the 2004 Decade of Behavior Research Award from the American Psychological Association, and the 2007 NASA Distinguished Public Service Medal.

Appendix H

Acronyms and Abbreviations

AAFES	Army and Air Force Exchange Service
ADC	Average daily consumption
ADL	Activities of daily living
AI	Adequate Intake
ALS	Amyotrophic lateral sclerosis
AMDR	Acceptable Macronutrient Distribution Range
AMEDD	Army Medical Department
AMS	Acute mountain sickness
ANS	Autonomic nervous system
APFT	Army Physical Fitness Test
ATP	Adenosine-5′-triphosphate
AUC	Area under the curve
BMD	Bone mineral density
BMI	Body mass index
BP	Blood pressure
CAM	Complementary and Alternative Medicine
CDR	Cognitive drug research
CFF	Critical flicker fusion
CGI	Clinical Global Impression scale
cGMP	Current good manufacturing practice
CHPPM	Center for Health Promotion and Preventive Medicine
CK	Creatine kinase
CNS	Central nervous system

CRT	Choice reaction time
CSFII	Continuing Survey of Food Intakes by Individuals
CVD	Cardiovascular disease
CYP2D6	Cytochrome P450 2D6
DEXA	Dual energy X-ray absorptiometry
DFE	Dietary Folate Equivalents
DHEA	Dehydroepiandrosterone
DHEA-S	the sulfated form of DHEA circulating in the body
DoD	Department of Defense
DRI	Dietary Reference Intake
DS	Dietary supplement
DSHEA	Dietary Supplement Health and Education Act
DSI EC	Dietary supplement information executive committee
DSID	Dietary Supplement Ingredient Database
DSNDCPA	Dietary Supplement and Nonprescription Drug Consumer Protection Act
DSVP	Dietary supplement verification program
EAR	Estimated Average Requirement
ECG	Electrocardiogram
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
ER	Emergency room
ERGO	Energy rich, glucose optimized
ESP	E-screener panel
FDA	U.S. Food and Drug Administration
FFD&C Act	Federal Food, Drug, and Cosmetic Act
GABA	Gamma-aminobutyric acid
GAS	Ginseng abuse syndrome
GI	Gastrointestinal
GRAS	Generally recognized as safe
HAS	High-altitude sickness
HDL	High-density lipoprotein
HFA	Health and Fitness Assessment
HMB	β-Hydroxy-β-methylbutyrate
HMG-CoA	3-Hydroxy-3-methylglutaryl coenzyme A
HPPI	Health Promotion and Prevention Initiative
HR	Heart rate

IARC	International Agency for Research on Cancer
ICW	Intracellular water
IGF-1	Insulin-like growth factor
IMQ	Index of Memory Quality
INR	International normalized ratio
IOM	Institute of Medicine
LLS	Lake Louise Score
MAJCOM	Major Command (USAF)
MAOD	Maximal accumulated oxygen deficit
MAOI	Monoamine oxidase inhibitor
MAP	Maximal aerobic power
MDRI	Military Dietary Reference Intake
MRCA	Market Research Corporation of America
MRI	Magnetic resonance imaging
MV/MM	Multivitamins/multiminerals supplements
NAS	National Academy of Sciences
ND	Not determined
NE	Niacin Equivalent
NEX	Navy Exchange Service
NF	National Formulary
NFCS	Nationwide Food Consumption Survey
NHANES	National Health and Nutrition Examination Survey
NHIS	National Health Interview Survey
NSORs	Nutritional Standards for Operational Rations
OASD(HA)	Office of the Assistant Secretary of Defense (Health Affairs)
ODS, NIH	Office of Dietary Supplements, National Institutes of Health
OGTT	Oral glucose tolerance test
OIG	Office of the Inspector General
P&T	Pharmacy and Therapeutics
PA	Physical activity
PCC	Poison control center
PGE_2	Prostaglandin E_2
PIF	Proteolysis-inducing factor
PKC	Protein kinase C
PSA	Prostate-specific antigen
PSG	Polysomnography
PUFA	Polyunsaturated fatty acid

RAE	Retinol Activity Equivalent
RDA	Recommended Dietary Allowance
RE	Retinol Equivalent
REE	Resting energy expenditure
RM	Reference material
RMG	Random movement generation test
RVIP	Rapid visual information processing
SBP	Systolic blood pressure
SF	Special Forces
sIgA	Secretory immunoglobulin A
SJS	Stevens-Johnson syndrome
SRT	Selective reminding test
TBW	Total body water
tHcy	Total plasma homocysteine
TNF-α	Tumor necrosis factor-alpha
TTCP	The Technical Corporation Program
UL	Tolerable Upper Intake Level
URTI	Upper respiratory tract infection
USACHPPM	U.S. Army Center for Health Promotion and Preventive Medicine
USAF	U.S. Air Force
USARIEM	U.S. Army Research Institute of Environmental Medicine
USDA	U.S. Department of Agriculture
USP	United States Pharmacopeia
USUHS	Uniformed Services University of the Health Sciences
VCO_2	Rate of elimination of carbon dioxide
V_E	Pulmonary ventilation during exercise
VO_2	Oxygen uptake
WAIS	Wechsler Adult Intelligence Scale
WHO	World Health Organization
WMS	Wechsler Memory Scale
WRAIR	Walter Reed Army Institute of Research

Appendix I

Glossary

Antihyperglycemic	An agent that counteracts high levels of glucose in the blood
Antioxidant	A substance that inhibits the destructive effects of oxidation
Beta-blockers	A class of drugs used for various indications, but particularly for the management of cardiac arrhythmias and cardioprotection after myocardial infarction. They block the action of endogenous catecholamines (epinephrine [adrenaline] and norepinephrine [noradrenaline] in particular) on β-adrenergic receptors, part of the sympathetic nervous system that mediates the fight-or-flight response
Botanicals	A drug, medicinal preparation, or similar substance obtained from a plant or plants
Codex Ebers	A famous Egyptian papyrus recording over 800 medical formulas
CYP2D6	Enzymes involved in the metabolism of xenobiotics in the body

Dietary supplement	A product (other than tobacco) that is intended to supplement the diet; contains one or more dietary ingredients (including vitamins, minerals, herbs or other botanicals, amino acids, and other substances) or their constituents; is intended to be taken by mouth as a pill, capsule, tablet, or liquid; and is labeled in the front panel as being a dietary supplement
Drug	The term *drug* means (A) articles recognized in the official United States Pharmacopeia, official *Homeopathic Pharmacopeia of the United States*, or official *National Formulary*, or any supplement to any of them; and (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (C) articles (other than food) intended to affect the structure or any function of the body of man or other animals; and (D) articles intended for use as a component of any articles specified in clause (A), (B), or (C). A food or dietary supplement for which a claim, subject to sections 403(r)(1)(B) and 403(r)(3) or sections 403(r)(1)(B) and 403(r)(5)(D), is made in accordance with the requirements of section 403(r) is not a drug solely because the label or the labeling contains such a claim. A food, dietary ingredient, or dietary supplement for which a truthful and not misleading statement is made in accordance with section 403(r)(6) is not a drug under clause (C) solely because the label or the labeling contains such a statement
Ergogenic aids	Any external influences that can positively affect physical or mental performance. These include mechanical aids, pharmacological aids, physiological aids, nutritional aids, and psychological aids. They may directly influence the physiological capacity of a particular body system, thereby improving performance; remove psychological constraints that detract from performance; or increase the speed of recovery from training and competition
Food	The term *food* means (1) articles used for food or drink for man or other animals, (2) chewing gum,

	and (3) articles used for components of any such article
Food additive	Any substance the intended use of which results or may reasonably be expected to result—directly or indirectly—in its becoming a component or otherwise affecting the characteristics of any food. This definition includes any substance used in the production, processing, treatment, packaging, transportation, or storage of food
GLUT-4	An important factor in the regulation of blood glucose, and the primary glucose transporter in skeletal muscle
Hyperinsulinemic	A condition in which the level of insulin in the blood is higher than normal. Caused by overproduction of insulin by the body. Related to insulin resistance
Hypocaloric	A diet characterized by a low number of dietary calories, usually 1,000–1,200 kcal/day
MedWatch	The U.S. Food and Drug Administration (FDA) Medical Products Reporting Program is an initiative designed both to educate all health professionals about the critical importance of being aware of, monitoring for, and reporting adverse events and problems to the FDA and/or the manufacturer and to ensure that new safety information is rapidly communicated to the medical community, thereby improving patient care. The purpose of the MedWatch program is to enhance the effectiveness of postmarketing surveillance of medical products as they are used in clinical practice and to rapidly identify significant health hazards associated with these products
Obese	For adults, an individual with a body mass index (BMI) greater than or equal to 30.0 kg/m^2
Overweight	An individual with a BMI of 25.0 to 29.9 kg/m^2

Pharmacovigilance	The pharmacological science relating to the detection, assessment, understanding, and prevention of adverse effects, particularly long-term and short-term side effects of medicines. Generally speaking, pharmacovigilance is the science of collecting, monitoring, researching, assessing, and evaluating information from health care providers and patients on the adverse effects of medications, biological products, herbal products, and traditional medicines with a view to identifying new information about hazards associated with medicines and preventing harm to patients
Phytates	Compounds present in plant foods that bind iron and may prevent its absorption
Rangers	A specially trained elite unit of the United States Army
Special Forces	Special Forces are highly trained and organized elite groups of soldiers who take part in covert warfare, reconnaissance, counterterrorism, and other specialized and dangerous missions. They include U.S. Army Special Forces, Navy SEALs (Sea, Air, Land) and Special Warfare Combatant Craft Crewmen, Air Force Combat Controllers and Pararescuemen
Thermogenics	Supplements that induce the production of heat

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Changes of tissue creatine concentrations upon oral supplementation of creatine-monohydrate in various animal species

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Abstract

Creatine is a nutritional supplement with major application as ergogenic and neuroprotective substrate. Varying supplementation protocols differing in dosage and duration have been applied but systematic studies of total creatine (creatine and phosphocreatine) content in the various organs of interest are lacking. We investigated changes of total creatine concentrations in brain, muscle, heart, kidney, liver, lung and venous/portal plasma of guinea pigs, mice and rats in response to 2–8 weeks oral creatine-monohydrate supplementation (1.3–2g/kg/d; 1.4 –2.8 % of dietary intake). Analysis of creatine and phosphocreatine content was performed by high performance liquid chromatography. Total creatine was determined as the sum of creatine and phosphocreatine. Presupplementation total creatine concentrations were high in brain, skeletal and heart muscle (10–22 μmol/g wet weight), and low in liver, kidney and lung (5–8 μmol/g wet weight). During creatine supplementation, the relative increase of total creatine was low (15–55% of presupplementation values) in organs with high presupplementation concentrations, and high (260–500% of presupplementation values) in organs with low presupplementation concentrations. The increase of total creatine concentrations was most pronounced after 4 weeks of supplementation. In muscle, brain, kidney and lungs, an additional increase ($p<0.01$) was observed between 2–4 and 2–8 weeks of supplementation. Absolute concentrations of phosphocreatine increased, but there was no increase of the relative (percentual) proportion of phosphocreatine (14–45%) during supplementation. Statistical comparison of total creatine concentrations across the species revealed no systematically differences in organ distribution and in time points of supplementation. Results suggest that in organs with low presupplementation creatine levels (liver, kidney), a major determinant of creatine uptake is an extra-intracellular concentration gradient. In organs with high presupplementation total creatine levels like brain, skeletal and heart muscle, the maximum capacity of creatine accumulation is low compared to other organs. A supplementation period of 2 to 4 weeks is

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necessary for significant augmentation of the creatine pool in these organs. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Total creatine; Nutritional supplement; Brain; Muscle; Heart; Kidney; Lung

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Introduction

Creatine is an aminoacid derivate synthesised in liver and pancreas at rates of 1–2 g per day [1]. Creatine is transported to muscle and brain and is there highly concentrated via an active, high affinity transmembrane transport process [2–4]. Together with the activity of creatine kinase and ATP / ADP, intracellular creatine and phosphocreatine represent a high energy buffering system with the aim to provide constant ATP supply [5].

While first studies with oral creatine supplementation have been already performed in the early 20th century [6,7], major interest in creatine as a nutritional supplement has evolved during the past 10 years. Main applications have been found in sports medicine [8–10] and in the treatment of various disease states: Oral supplementation of high doses of creatine-monohydrate has been shown to augment muscle total creatine concentrations [11] and to improve muscle performance [12]. In addition, cardioprotective [13,14] and anticancer effects [15] of creatine have been studied. Presently the role of creatine as a neuroprotective substance is intensively investigated in vitro, and in animal experiments. In particular, protective effects in animal models of Huntington's disease and amyotrophic lateral sclerosis [16,17,18], and in experimental sets of hypoxic-ischemic cell damage [19–24] have been demonstrated. Finally, both in vitro experiments and patient studies suggest beneficial effects of creatine in muscular dystrophies and metabolic myopathies [25–27].

The studies on dietary creatine supplementation have been carried out with varying protocols differing in dosage and duration of creatine supplementation, but in most studies neither the total creatine content in the tissues of interest (brain, skeletal and heart muscle) nor the relative distribution of creatine in the body's organs in response to creatine supplementation has been studied in detail.

The aim of this study was to investigate changes of total creatine concentrations in response to dietary creatine supplementation. For this purpose a 2–8 week protocol of high-dose oral creatine-monohydrate supplementation was applied in three animal species (guinea pig, mouse, rat), and the concentrations of total creatine (creatine and phosphocreatine) in the various organs and tissues were measured.

Materials and methods

Animals

Experiments were carried out in 3 animal species: rat (Him:OFA stock), mouse (Him:OF1) and guinea pig (Him:DH). All animals were bred at the Institute for Laboratory Animal Science and Genetics, Himberg, Austria. The experiments were performed at the experimental unit of the same facility. The animals were kept in groups of 3–5 animals in cages made of Macrolon and filled with soft wood bedding. From all species only female animals were included in the experiment, as male mice exhibit aggressive behaviour when kept together in the same cage. A standard diet (rats and mice: Altromin 1324, guinea pigs: Altromin 3022) was available ad libitum. Room temperature was 22±1°C, relative humidity 50±10 %. Ventilation with 100 % fresh air caused an air change 15 times per hour. The room was illuminated with artificial light at an intensity of about 200 lux from 7–5 p.m.

Creatine supplementation and feeding protocols

All animals were 6 weeks old when the feeding experiment was started. Creatine was supplemented as creatine-monohydrate (Sigma C3630) dissolved in the animal's drinking water.

Rates of basal metabolism and conversion of compounds in warm-blooded animals are proportional to body surface, i.e. the smaller the organism, the larger its relative body surface and the more intense its metabolism [28]. According to current principles of animal extrapolation [29], dosages for animals were chosen such that they were equivalent to 400 – 600 mg creatine-monohydrate per kg human body weight. Such dosages are applied in athletes and in patients with inborn errors of creatine synthesis [30] with the aim to augment the muscle and body creatine pool. Under these prerequisites, to mice and rats, a dosage of 2 g per kg body weight per day (i.e. 1.4 % and 2.4 % of daily nutritional intake) was given. To guinea pigs, due to physiological low water consumption and limited water-solubility of creatine-monohydrate, a dosage of 1.3 g creatine-monohydrate per kg body weight per day (i.e. 2.8 % of daily nutritional intake) was given. Extrapolating to humans, this dosage corresponds to 400 mg per kg human body weight.

In mice and rats, saccharose (1%, i.e. 1.6 and 0.8 g per kg body weight per day) was added supplementary to the drinking water, as in previous experiments we have observed that animal's consumption of drinking water supplemented with creatine-monohydrate alone is insuffiencent, but normalised when taste is improved by the addition of saccharose. In guinea pigs, in accordance with their essential requirements, ascorbic acid (Richter Pharma AG, Wels) was added to the drinking water. Consumption of drinking water supplemented with creatine-monohydrate alone was sufficient, and – unlike in mice and rats - saccharose supplements were not required.

Creatine-monohydrate was supplemented during 2-, 4-, and 8-week periods. Drinking water supplemented with creatine-monohydrate in the supplementation groups was removed prior to anaesthesia of the animals.

Five animals were assigned to each supplementation group. Five additional animals were assigned to the respective control groups, which received no creatine supplementation. In mice and rats, saccharose was given both in the supplemented and in the unsupplemented groups. 8-week supplementation was started at the beginning of the experiment, while the 4- and 2-week supplementation was started after 4 and 6 weeks of the usual diet. With this feeding protocol, water consumption was within the expected normal range for all animals, and there were no differences in weight gain among the animals subjected to different periods of creatine supplementation.

Preparation of organs/tissues

In rats and guinea pigs, venous blood was taken from the retroorbital venous plexus under ketamin-xylazine anaesthesia (Ketalar: Parke-Davis GesmbH, Berlin, FRG; Rompun: Bayer

AG, Leverkusen, FRG). Subsequently an overdose of ketamin-xylazine was administered intracardially. After the vital reflexes had ceased to exist, the abdominal wall was dissected and blood was taken from the portal vein. In mice, venous blood was taken from the retroorbital venous plexus under ether anaesthesia (Merck KGaA, Darmstadt, FRG). Blood was collected into EDTA-K_2 vials. Subsequently the animals were sacrificed by cervical dislocation. In the following order, the skeletal muscle (quadriceps femoris and tensor fasciae latae), both kidneys, liver, lungs, heart and the brain were dissected and immediately frozen in liquid nitrogen and stored at –80 °C until the chemical analysis was performed. Blood was centrifuged at 5°C for 10 min and plasma was frozen like the other tissue samples.

In mice, the range of time delay between neck dislocation and freezing the organs was 40 sec for the first dissected organ (skeletal muscle) and 120 – 140 sec for the last dissected organ (brain). In rats and guinea pigs, an additional 30 – 40 sec time delay was due to the collection of blood from the portal vein performed prior to organ dissection.

Extraction of tissue samples

Extraction was done as previously described [31] by homogenising in a glass tissue homogeniser with ice cold 0.5 M perchloric acid ($HClO_4$) containing 0.5 mM ethylene glylcolbis (beta-aminoethyl-ether) tetraacetic acid (EGTA) in a ratio of 200 mg per ml of acid solution. After 5 minutes on ice, the acid extract was centrifuged at 14.000 g for 3 minutes, and the clear supernatant was immediately neutralised with 0.4 M K_2CO_3 to pH 7.0, mixed well and kept at 4 °C for 5 minutes. The neutralised supernatant was centrifuged for 5 minutes at 10.000 g to remove the insoluble potassium perchlorate and the clear supernatant was immediately frozen at – 80 °C until subjected to high performance liquid chromatography (HPLC) analysis.

Chromatographic setting

Total creatine (creatine and phosphocreatine) was measured with a Hewlett Packard HP1050 series, equipped with quaternary pump and UV detector, a Marathon autosampler and a 50 μl sample loop (Spark, Holland). Separation of creatine and phosphocreatine was performed on a Hypersil ODS (250 mm × 4 mm ID, 5 μm) and C18 guard (25 mm × 4.6 mm) column. The mobile phase, freshly prepared prior to analysis, consisted of 100 mM NaH_2PO_4, 6 mM tetrabutylammonium-hydrogensulfate pH 6.0 with 1 N NaOH. The isocratic run was maintained at 1.0 ml/min and the UV detector was set at 210 nm. The complete analysis took 10 minutes for each sample.

Calculations and statistical methods

Total creatine concentrations were calculated as the sum of creatine and phosphocreatine.

One-way analysis of variance (ANOVA) was used for statistical comparison between the basal and the supplemental total creatine concentrations in the respective organs within the same animal species. Tukey's post-hoc test for multiple comparisons was used for determination of significant differences between the basal and the supplemental total creatine concentrations. Student's t-test was used for statistical comparison of basal and supplemental total creatine concentrations in the respective organs across the animal species. The level of statistical significance was set at $p<0.01$. Results of total tissue creatine concentrations are given as mean values ± SD. The relative increase of total tissue creatine concentrations during creatine supplementation is given in percent as compared to the basal (presupplementation) total creatine concentrations. All statistical analyses were performed using the SPSS (8.0) and Excel 97 software.

Results

Changes of total creatine concentrations over the time period of creatine-monohydrate supplementation

Presupplementation total creatine concentrations were high in brain, skeletal and heart muscle (10–22 μmol/g wet weight), and low in liver, kidney and lung (5–8 μmol/g wet weight). During creatine supplementation, the relative increase of total creatine was low (15–55% of presupplementation values) in organs with high presupplementation concentrations, and high (260–500% of presupplementation values) in organs with low presupplementation concentrations (table 1). The increase of total creatine concentrations was most pronounced after 4 weeks of supplementation. In muscle, brain, kidney and lungs, an additional increase ($p<0.01$) was observed between 2–4 and 2–8 weeks of supplementation (figure1).

Changes of the phosphocreatine fraction

The basal (presupplementation) percentage proportion of phosphocreatine in the total creatine fraction was 14 – 45%. Upon oral supplementation of creatine-monohydrate, the percentage proportion of phosphocreatine remained constant or even decreased in most organs, while the percentage proportion of free creatine (calculated as the difference between total and phosphocreatine) significantly increased during the time course of supplementation. In terms of absolute values, there was a significant increase of phosphocreatine in the livers and in most plasma samples of all animal species, and in the brains and skeletal muscles of guinea pigs. In the remaining organs, the absolute values of phosphocreatine concentrations were rather inconsistent and statistical significance of changes could not be shown (table 1).

Discussion

In the present study we examined the effects of high dosage oral creatine-monohydate supplementation over different periods of time (2, 4, and 8 weeks) on total creatine concentrations in the animal species guinea pig, mouse, and rat. We observed no significant differences in body weight in animals with and without creatine supplementation and in animals that received creatine-monohydrate supplementation over short (2 weeks) and long periods (8 weeks). Therefore the side effect of increased body weight (0.7 to 2 kg) after 1–2 weeks of 20–25 g creatine/day as reported in humans [10,32] was not observed in the animals studied here.

Two types of organs were seen to differ in basal (presupplementation) total creatine concentrations and in the degree of relative increase during supplementation: Type one with high basal total creatine concentrations (10–22 μmol/g wet weight) included skeletal muscle, heart muscle and brain, and type two with significantly lower basal total creatine concentrations (5–8 μmol/g wet weight) included liver, kidney and lung. Regarding the degree of rela-

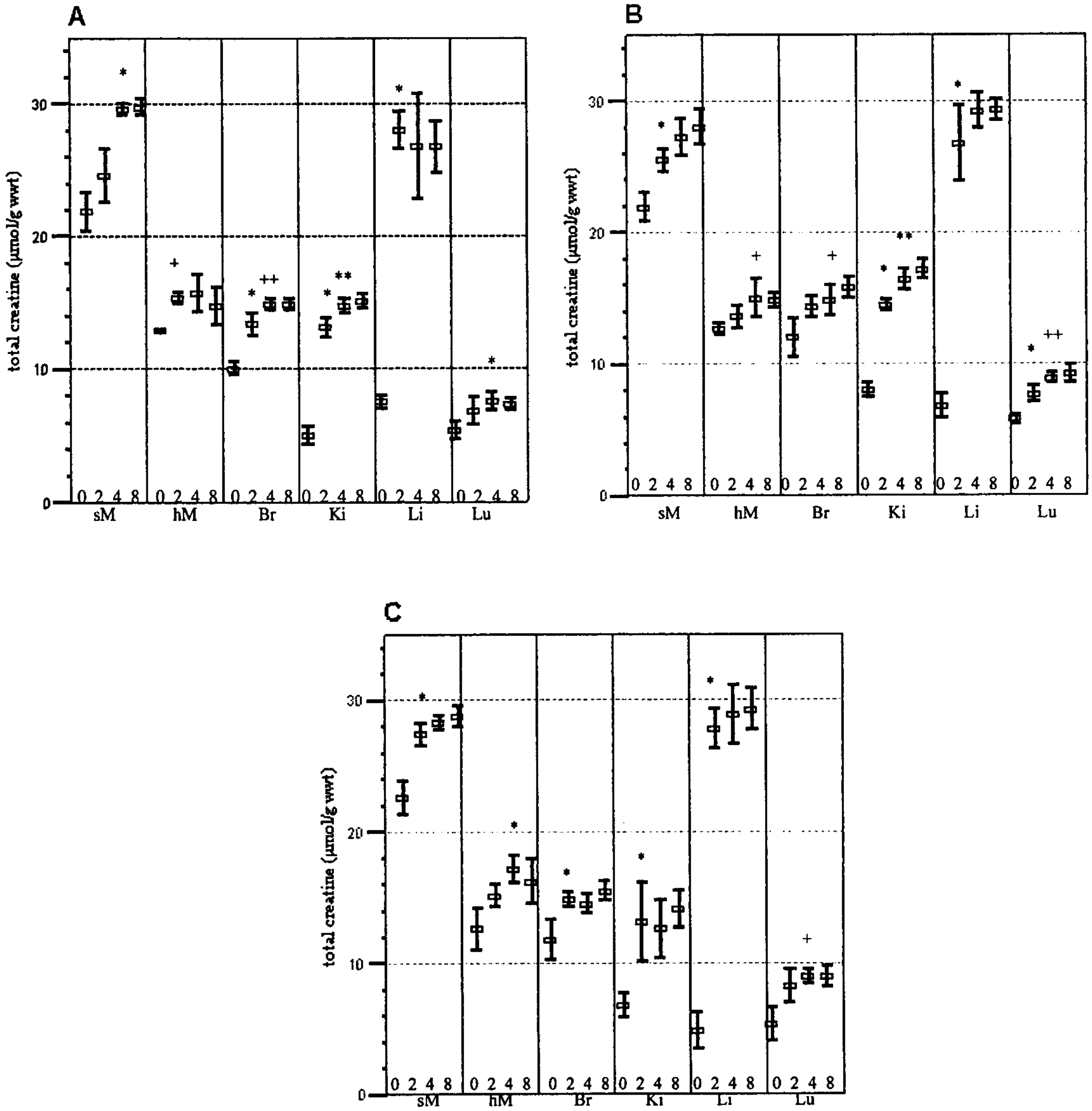

Fig. 1. Changes of totale creatine concentrations over the time period of creatine-monohydrate supplementation. Total creatine concentrations are given as mean ± SD on the vertical axis in guinea pig (A), mouse (B), and rat (C). Supplementation period in weeks is given as 0,2,4,8 on the horizant axis. Statistical significant change compared to presupplementation (0 week) values is marked with * (p<0.001), $^+$ (p<0.01). Statistical significant changes compared to the 2 week supplementation values is marked with ** (p<0.001) and $^{++}$ (p<0.01). sM = skeletal muscle, hM = heart muscle, Br = brain, Ki = kidney, Li = liver, Lu = lung.

tive increase of total creatine concentrations during supplementation, we found a striking difference between these two types of organs. In particular, the relative increase was low (increase by 15–55 % of presupplementation values) in organs and tissues with high basal total creatine concentrations (brain, skeletal and heart muscle), while the relative increase was high in the liver (increase by 260–500 % of presupplementation values) with lowest basal total

Table 1
Total creatine (TCr9 and phosphocreatine (PCr) concentrations prior and during supplementation of creatine-monohydrate supplementation in organs and plasma of guinea pigs, mice, and rats

		0 week- basal levels			*2 week- supplementation levels*			*4 week- supplementation levels*			*8 week- supplementation levels*		
		guinea pig	mouse	rat	guinea pig	mouse	rat	guinea pig	mouse	rat	guinea pig	mouse	rat
sM	*TCr*	21.9 ± 1.31	22.0 ± 0.97	22.6 ± 1.13	24.6 ± 1.84	25.6 ± 0.74	27.5 ± 0.75	**m* 29.6 ± 0.34	27.3 ± 1.24	28.3 ± 0.48	***+ 36 %*** 29.8 ± 0.53	***+ 27 %*** 28.0 ± 1.19	***+ 27 %*** 28.8 ± 0.72
	PCr	3.11 ± 0.35 14 %	4.52 ± 2.22 21 %	3.61 ± 0.74 16 %	4.97 ± 2.11 20 %	5.43 ± 0.50 21 %	5.18 ± 0.64 19 %	6.43 ± 1.34 22 %	4.85 ± 0.86 18 %	4.52 ± 0.93 16 %	+ 7.02 ± 0.75 24 %	3.7 ± 1.61 13 %	5.41 ± 0.63 19 %
hM	*TCr*	12.9 ± 0.10	12.7 ± 0.39	12.6 ± 1.39	**m* 15.4 ± 0.34	13.6 ± 0.78	15.1 ± 0.74	15.7 ± 1.27	15.1 ± 1.27	17.2 ± 0.93	***+ 15 %*** 14.8 ± 1.25	***+ 17 %*** 14.9 ± 0.47	***+ 28 %*** 16.2 ± 1.5
	PCr	3.35 ± 0.75 27 %	4.96 ± 3.55 39 %	3.4 ± 0.90 27 %	4.07 ± 0.75 26 %	4.5 ± 0.64 33 %	3.02 ± 0.78 20 %	4.74 ± 0.74 30 %	4.16 ± 0.65 28 %	4.01 ± 0.39 23 %	3.53 ± 1.21 24 %	3.06 ± 0.73 21 %	3.8 ± 1.01 23 %
Br	*TCr*	10.1 ± 0.45	12.0 ± 1.34	11.9 ± 1.36	13.4 ± 0.79	14.4 ± 0.68	14.9 ± 0.51	14.9 ± 0.38	14.9 ± 1.05	14.6 ± 0.62	***+ 54 %*** 15.6 ± 0.49	***+ 32 %*** 15.9 ± 0.68	***+ 30 %*** 15.5 ± 0.61
	PCr	3.11 ± 0.3 31 %	3.83 ± 1.29 32 %	4.31 ± 0.93 36 %	+ 4.46 ± 0.73 33 %	5.68 ± 1.03 40 %	5.39 ± 0.30 36 %	* 5.38 ± 0.18 36 %	5.45 ± 2.27 37 %	5.86 ± 0.61 40 %	* 6.09 ± 0.40 39 %	5.24 ± 0.53 33 %	+ 6.0 ± 0.57 39 %
Ki	*TCr*	*+m, *r* 5.0±0.60	8.1 ± 0.51	6.9 ± 0.81	13.1 ± 0.62	14.5 ± 0.36	13.2 ± 2.65	14.8 ± 0.47	16.5 ± 0.73	12.6 ± 1.95	***+ 204 %*** 15.2 ± 0.49	*+r* ***+ 112 %*** 17.2 ± 0.63	***+ 104 %*** 14.1 ± 1.27
	PCr	2.25 ± 0.33 45 %	2.2 ± 0.33 27 %	1.92 ± 0.69 28 %	5.52 ± 4.03 42 %	3.79 ± 0.30 26 %	3.75 ± 1.69 29 %	4.38 ± 0.66 30 %	3.65 ± 0.74 22 %	2.88 ± 1.76 23 %	4.23 ± 1.11 28 %	3.37 ± 1.06 20 %	3.54 ± 0.77 25 %
Li	*TCr*	**r* 7.5 ± 0.47	6.8 ± 0.82	4.9 ± 1.22	28.0 ± 1.23	26.8 ± 2.57	27.8 ± 1.39	26.8 ± 3.54	29.3 ± 1.17	28.9 ± 2.0	***+ 257 %*** 26.8 ± 1.76	***+ 331 %*** 29.3 ± 0.72	***+ 500 %*** 29.4 ± 1.39
	PCr	3.0 ± 0.99 40 %	2.13 ± 0.34 31 %	2.06 ± 0.50 42 %	* 8.43 ± 0.36 30 %	* 6.69 ± 1.19 25 %	* 7.52 ± 1.34 27 %	+ 7.23 ± 1.97 27 %	* 6.95 ± 0.83 24 %	+ 6.98 ± 1.76 24 %	* 8.23 ± 2.04 31 %	* 7.51 ± 1.34 26 %	+ 6.36 ± 2.28 22 %
Lu	*TCr*	5.4 ± 0.56	5.8±0.32	5.4 ± 1.14	6.9 ± 0.89	7.7 ± 0.54	8.3 ± 1.15	**m, *r* 7.6 ± 0.59	8.9 ± 0.31	9.06 ± 0.48	*+m, *r* ***+ 35 %*** 7.3 ± 0.35	***+ 60 %*** 9.3 ± 0.62	***+ 67 %*** 9.04 ± 0.72
	PCr	1.18 ± 0.21 22 %	2.47 ± 0.26 42 %	1.46 ± 0.76 27 %	1.62 ± 0.97 24 %	2.44 ± 0.77 32 %	1.12 ± 0.66 14 %	1.37 ± 1.09 10 %	2.44 ± 0.29 27 %	1.76 ± 0.98 20 %	0.73 ± 0.23 10 %	2.14 ± 0.52 23 %	0.9 ± 0.29 10 %
pP	*TCr*	0.33 ± 0.09	0.21±0.08	0.36 ± 0.07	1.04 ± 0.15	1.08 ± 0.22	1.16 ± 0.07	1.0 ± 0.15	1.16 ± 0.09	1.11 ± 0.11	***+ 242 %*** 1.13 ± 0.13	***+ 452 %*** 1.16 ± 0.2	***+ 231 %*** 1.19 ± 0.1
	PCr	0.09 ± 0.03 27 %	0.07 ± 0.03 33 %	0.1 ± 0.01 28 %	0.2 ± 0.08 19 %	* 0.28 ± 0.07 26 %	+ 0.33 ± 0.08 28 %	0.16 ± 0.16 16 %	* 0.29 ± 0.04 25 %	0.23 ± 0.09 21 %	0.25 ± 0.06 22 %	* 0.35 ± 0.07 30 %	* 0.35 ± 0.09 30 %
vP	*TCr*	0.18 ± 0.05	n.d.	0.3 ± 0.06	0.9 ± 0.06	n.d.	0.84 ± 0.11	0.82 ± 0.06	n.d.	0.91 ± 0.09	***+406%*** 0.91 ± 0.02	n.d.	***+217 %*** 0.95 ± 0.05
	PCr	0.06 ± 0.01 33 %	n.d.	0.11 ± 0.03 37 %	* 0.31 ± 0.07 34 %	n.d.	0.2 ± 0.06 24 %	0.16 ± 0.05 20 %	n.d.	0.19 ± 0.11 21 %	+ 0.22 ± 0.08 24 %	n.d.	0.21 ± 0.06 22 %

Concentrations are given as mean ± SD in µmol/g wet weight (organs), and in µmol/l (plasma). Statistically significant differences are marked with * ($p<0.001$), and with $^+$ ($p<0.01$). Interspecies differences to mice and rats are marked with m, and r. PCr is given as absolute concentrations (first line), and as percentual proportion of TCr (%, second line). In the 8 week supplementation group, the relative increase of TCr is additionally given in percent as compared to the 0-week (presupplementation) group (+%). n = 5 per animal species and group. sM = skeletal muscle, hM = heart muscle, Br = brain, Ki = kidney, Li = liver, Lu = lung, pP = portal plasma, vP = venous plasma; n.d. = not determined.

creatine concentrations, and in the kidney (increase by 100–200 % presupplementation values) with intermediate basal total creatine concentrations. Comparing our results with the literature, on the one hand the high increase in total creatine levels of liver and kidney is also confirmed by the results of Horn et al [33] who found a comparable high increase of total creatine levels in kidney (+240%) and livers (+400%) of rats upon chronic dietary creatine feeding. On the other hand, the low increase of creatine concentrations in brain and muscle, is confirmend by the results of previous human studies, demonstrating a maximum 20 % increase of total creatine levels in skeletal muscle [8–11,34] and a maximum 5–15 % increase of total creatine levels in the brain [35].

In creatine requiring organs like muscle and brain, creatine uptake is mainly effected by the active creatine transporter system [2]. Basal total creatine concentrations are high and further augmentation of the total creatine content e.g. by excess dietary creatine uptake is limited by the given maximum activity of the creatine transporter system. In contrast, liver and kidney are non creatine requiring organs lacking the active creatine transporter system and thus displaying only low tissue creatine levels under basal (presupplementation) conditions. The enormous increase of creatine upon supplementation as seen in these organs suggests that the intra–extracellular creatine concentration gradient is another important determinant of creatine uptake especially in the organs with low activity of the creatine transport system. However, while in the liver the relative increase of creatine accumulation was directly comparable to the changes in portal plasma, thus reflecting a passive, gradient dependent process only, the less pronounced changes observed in the kidney indicate an additional saturable, transporter mediated uptake process [2,36] which might play a role in the tubular reabsorption of creatine [37].

Lung tissue belongs to the type of organs with rather low basal creatine levels, but the degree of their relative increase during supplementation was rather low (30–60%). Lung is an organ rich in blood, and the observed relative increase of total creatine seems rather due to the creatine content in the blood than to processes of active creatine uptake like in brain and muscle tissue.

Over the time period of supplementation, in all organs and in all species the most pronounced increase of tissue total creatine concentrations was found within the first 4 weeks of supplementation. An additional significant increase between 2–4, and 2–8 weeks of supplementation was found in some organs (mainly brain and kidney) of guinea pigs and mice. A similar pattern of delayed increase in total creatine content was observed in the brain of a patient with an inborn error of creatine biosynthesis upon long term high dosage creatine supplementation [30]. Downregulation of the creatine transporter activity in the presence of high extracellular creatine concentrations and passive creatine transport effected by an intra-extracellular gradient may represent the main cause of this delayed uptake.

In humans, ingestion of carbohydrates has been shown to augment creatine uptake and accumulation in muscle tissue [38,39]. However, this effect was only evident when carbohydrates were given as a multiple dosage (400 g) of the creatine-monohydrate (20 g) supplement. In our study, the amount of carbohydrate added to mice's and rat's diet was comparably low (1.6 and 0.8 g saccharose per kg body weight per day, vs. 2 g per kg body weight per day creatine-monohydrate) and therefore an influence of the saccharose supplement on creatine uptake in these animal's organs and tissues is unlikely.

Compared across the species, some animals (mainly guinea pigs) had statistically different concentrations of total creatine in various organs. However, these changes were not systematically present neither in a particular organ nor in a particular period of creatine supplementation. As creatine-monohydrate containing drinking water was offered to the animals until shortly before they were sacrificed, contamination of organs with creatine rich blood (e.g. of liver and lung) is possible. This may explain both the varying SD in the total creatine concentration (mainly of liver) and the unsystematical interspecies differences. In contrast to these quantitative differences, qualitative patterns of changes in total tissue creatine concentrations over the time period of creatine supplementation were similar in all species investigated. It is therefore plausible that similar patterns may be expected also in humans upon comparable dosages of creatine supplementation.

The total intracellular creatine concentration is an essential component of energy metabolism since it determines the concentration of the available high energy phosphate, phosphocreatine. Edström et al [40] demonstrated a 35–62% phosphorylation degree in different muscles from rats and guinea pigs. In human muscle 60 % of total creatine is present as its high energy derivate phosphocreatine, and an enhancement of postexercise phosphocreatine resynthesis has been shown after oral creatine supplementation [41]. In our experiments, the basal (presupplementation) relative proportion of phosphocreatine was comparably low accounting for minimum 14 % and maximum 45% of the total creatine fraction, and no systematic increase of the phosphocreatine fraction was observed during creatine supplementation. Phosphocreatine is a high energy phosphate compound [42,43] prone to degradation during post mortem organ harvesting and preanalytical sample preparation. The low percentual proportion of phosphocreatine in the total creatine fraction as observed in our experiments and the broad range of absolute concentrations as expressed in the high standard deviation values, might be due to methodological problems in the measurement of phopshocreatine, e.g. influence of hypoxia during anaesthesia, direct influence of the anaesthetic agents on the metabolism of high energy phosphates and delayed freezing as described in the dissection protocol. For reliable assessment of changes in phosphocreatine concentrations in response to creatine supplementation, separate series of experiments using sample preparation techniques that preserve the integrity of high-energy phosphate compounds for analysis, are needed.

Conclusion

Essentially, in the present study we could show that long-term high-dosage oral creatine supplementation leads to a significant augmentation of total tissue creatine concentrations in various animal species. Unlike kidney and liver, the capacity of further augmentation of the basal (presupplemental) tissue creatine pool is limited in brain and muscle tissue. Therefore, in studies aimed to demonstrate neuroprotective and ergogenic effects of oral creatine supplementation, total creatine concentrations should be measured and evidence of significant changes in the organs of particular interest should be provided. Under the current assumption that an absolute increase of the tissue creatine pool is essential in achieving therapeutic benefits, long-term supplementation rather than application of single dosages of creatine is warranted.

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